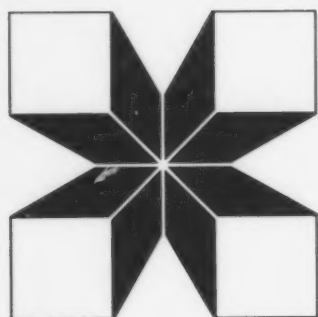


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C A N A D A

LEISHMANIASIS CONTROL STRATEGIES

A CRITICAL EVALUATION OF
IDRC-SUPPORTED RESEARCH

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This series includes meeting documents, internal reports, and preliminary technical documents that may later form the basis of a formal publication. A Manuscript Report is given a small distribution to a highly specialized audience.

La présente série est réservée aux documents issus de colloques, aux rapports internes et aux documents techniques susceptibles d'être publiés plus tard dans une série de publications plus soignées. D'un tirage restreint, le rapport manuscrit est destiné à un public très spécialisé.

Esta serie incluye ponencias de reuniones, informes internos y documentos técnicos que pueden posteriormente conformar la base de una publicación formal. El informe recibe distribución limitada entre una audiencia altamente especializada.

Leishmaniasis control strategies



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June 1992

Leishmaniasis control strategies: A critical evaluation of IDRC-supported research

Proceedings of a workshop held in Mérida, Mexico, November 25–29, 1991, sponsored by the International Development Research Centre, in collaboration with the Universidad Autónoma de Yucatán (UADY) and the Universidad Peruana Cayetano Heredia (UPCH)

Edited by
Pandu Wijeyaratne, Tracey Goodman
and Carlos Espinal

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FOREWORD

Leishmaniasis is considered to be a disease of the poor, and it certainly has been shown to be a major obstacle to "development" in endemic countries. Currently, epidemics of visceral leishmaniasis (Kala azar) are raging in Sudan and India with at least 60,000 deaths. A recent press release from the World Health Organization¹ highlights further that "Leishmaniasis is one of the most rapidly spreading of all parasitic diseases: 12 million people are infected in 80 countries in which 350 million persons are at risk. In some endemic countries it is increasingly recognized as an opportunistic infection in AIDS patients".

As a funding agency, IDRC has been unique in supporting community-based, applied field research on tropical diseases incorporating ecological, social, cultural and economic dimensions. This interdisciplinary approach has been considered to be very successful and complements the more bio-medical emphasis of other agencies. From an IDRC perspective, a major neglected disease, such as leishmaniasis, which affects the more remote areas of developing countries, provides an excellent entry point to approach the overall health and well-being of communities.

Since the mid-1980s IDRC has supported 15 community-based applied research projects on leishmaniasis in Jordan, Ethiopia, Kenya, Tunisia, Mexico, Costa Rica, Colombia, Peru and Brazil. IDRC's strategy of support is derived from the recommendations of a 1987 leishmaniasis workshop in Ottawa which was convened by IDRC, in association with the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Universidad Peruana Cayetano Heredia (UPCH, Peru). As many of these IDRC-supported projects are now approaching first or second phase completion, a main objective of the Mérida Workshop was to critically review and evaluate the research results in light of their application to the prevention and control of leishmaniasis. The 56 participating researchers, scientists, policy-makers and international representatives (such as TDR WHO, PAHO and the Liverpool School of Tropical Medicine) were challenged to explore in detail "How close are we to implementing feasible preventive and control measures to protect communities at risk?"

The workshop began with the presentation of four orientation papers highlighting the expanding context of leishmaniasis research. The papers included in this report, introduce general topics related to control strategies such as the need for an interdisciplinary view of endemic diseases, the use of ecological and environmental (eco-epidemiological) methods, community participation, and health policy and delivery services for leishmaniasis. In each case, the importance of policy implementation is stressed as a key factor for control, particularly with respect to the mobilization of financial and human resources.

¹ WHO/24, 14 April 1992.

Following the thematic orientation, attention at the meeting turned to the intensive review and critical evaluation of the results of 15 IDRC-supported leishmaniasis research projects. Considerable time was devoted to this session which aimed to answer two key questions for present and future leishmaniasis control programs: "What have we learned and where do we go from here?"

The collection of scientific papers reporting on research project results presented at the meeting and contained in this report deal with a broad range of subjects from diverse disciplinary perspectives. Results of visceral, cutaneous and mucocutaneous leishmaniasis research are discussed extensively. Specific topics covered include ecological observations, clinical and field epidemiology, the impact of migration and resettlement, and the identification of community and environmental risk factors.

Findings which generated considerable interest with regards to implementing control strategies include:

- the active participation of the community in early detection and reporting of cases (Peru; Colombia);
- incrimination of livestock as natural reservoirs of infection and implications to human infection and prevention/control (Kenya);
- simplified immunodiagnosis for Chiclero's ulcer (Mexico) and cutaneous and mucocutaneous leishmaniasis (Peru; Colombia);
- innovative health education and environmental approaches to prevent domiciliary/peri-domiciliary transmission (Costa Rica);
- reduction of sand-fly biting of people through appropriate forestation (eucalyptus and neem) and plant use (Kenya; Peru);
- rapid incrimination of vectors via blood-meal identification (Peru);
- methods for rodent reservoir control (Tunisia; Jordan);
- social, economic and behavioral factors related to vector contact, particularly migration and resettlement (Ethiopia), economic activities such as mining, forestry and farming and the corresponding increased pathogenicity observed in younger individuals;
- the development of predictive mathematical models for cutaneous and mucocutaneous leishmaniasis in migrant communities (Peru; Colombia);
- complexity of the problem as it affects many areas in different continents with different ecology, parasite species, costs and availability of drugs, etc.

The presentation of scientific papers was followed by a series of five brief panel discussions. These panel discussions encouraged participants to grapple with pragmatic issues such as appropriating technology for leishmaniasis control and adapting leishmaniasis treatment to peripheral health centres and communities. Other panels explored immunological considerations for control, how leishmaniasis is addressed nationally as a public health problem, and whether vector and reservoir control is possible.

The final, and perhaps most important session of the workshop, was dedicated to four working groups. The aim of the working groups was to incorporate the knowledge generated during the previous sessions and address the following issues:

1. The role of the community in prevention and control;
2. Technology, availability and transfer for leishmaniasis
3. Regional patterns of leishmaniasis as a basis for action, and
4. Surveillance systems for leishmaniasis control.

A record of the working group discussions and recommendations are included in this manuscript report.

It was strongly agreed by all that the workshop was highly successful in accomplishing its aims and objectives. The opportunity to bring together a core network of leishmaniasis researchers to share results and plan for the future proved exciting and simulating. There is now a tremendous challenge and commitment to forge ahead together and to follow-up national projects with a multi-country network of intervention/control trials, based on the wealth of information generated.

Looking to the future, and as a first step towards establishing integrated control (primarily, ecological and community intervention) approaches, a project development workshop is to be held on July 20-25, 1992 in Cali, Colombia, hosted by two Colombian institutions, CIDEIM and CIMDER. Three country teams, Mexico, Costa Rica and Colombia (with participants from Tunisia and Peru in attendance) will attend the workshop with their already developed proposals to finalize details for a closely linked project network. It is anticipated that WHO/TDR and PAHO will collaborate with IDRC in the development, support of field implementation, and monitoring of this pioneering and most important venture in endemic disease control. It is hoped that this model network of similar but complimentary projects will not only address leishmaniasis control but more importantly the overall health and well being of the more peripheral communities of endemic countries, through leishmaniasis as the entry point.

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It is with a note of sadness that it should be noted that, just as with the 1987 meeting, the plans for this gathering had to be moved from the originally proposed venue of Lima, Peru and the initial hosts; Instituto Medicina Tropical "Alexander Von Humboldt", of Universidad Peruana Cayetano Heredia whose local organizer Luis Guevara had put in considerable organizational efforts, with his team.

Administrative and travel arrangements of international workshops at short notice are no easy task and I would like to acknowledge the great help of Angie Anton, Andree Lalonde and Julie Cousineau for their efficient organizational support, and to Carlos Espinal for his great help in planning and technical assistance with manuscripts and workshop presentations.

A final word of gratitude and deep appreciation goes to my colleague Tracey Goodman for her enormous support in the preparation and conduct of the workshop and in the single handed preparation of these proceedings for publication.

P.W.

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Creating and Sharing Knowledge for Action: Towards a New Way of Seeing the Problem of Endemic Diseases

Christina H. Zarowsky¹

I was going to call this paper "Can Anthropology, Ecology, Immunology, Political Science and Epidemiology Fit in One Useful Sentence?", but I discovered that they barely fit on the same page. Instead I decided to use my position as both a physician and a representative of a research funding agency to offer a critique of physicians and funding agencies. I would like to challenge a prevailing view of science as a neutral, objective enterprise of cooperation among equals from various research disciplines. The hope is that this will encourage us to rethink the way we see the problem of endemic diseases, so that research from different disciplines can be more effectively shared and coordinated.

I would like to start with a quote from a paper by Dona Lee Davis (1986 quoted with permission of the author), a North American anthropologist studying health conditions among North American women. In this case the condition is menopause, and the women are residents of a fishing village in Newfoundland, Canada's easternmost province. There is no link to leishmaniasis. However, as the author indicates in her paper, this vignette illustrates several of the issues we will be grappling with over the next few days, such as:

- the difficulty of communicating across cultural barriers, whether barriers of language, gender or occupation;
- the tension between wanting to quantify things that are difficult to measure (in this case, how women perceive menopause), and the need to acknowledge and explore their complexity.

The following exchange is between the researcher, trying to administer a questionnaire designed to be both quantitative and cross-culturally valid, and one respondent. It covers a single question ("R" refers to the researcher; "W" refers to the Harbour woman being interviewed).

- R: *To what extent do you have worries and problems about your work about the house? Are you: 1. especially worried, 2. quite worried, 3. not so worried, 4. almost not at all worried, or 0. not relevant.*
- W: *Which do you mean, dear? Worry or problems? You can have one without the other, you know. Mabel over in Crow Cove...*
- R: *This is about you. (Repeat question and answer items 1 - 4, 0).*
- W: *Well, my dear, why you ask that is beyond me, but I do tells you,*

¹ Health Sciences Division, International Development Research Centre (IDRC), Ottawa, Canada.

I do worry some awful. To tell the truth, I worry more than most, always have.... [I] got it from my own mother, her nerves, you see....

R: What about worry over your work in the house?

W: Tell me again what I'm supposed to say?

R: (Repeat question and answer items 1 - 4, 0).

...

W: What's the difference between "quite" and "not so" worried?

R: The quite means you're more worried than the "almost not at all" does.

W: That's not how we mean it, my dear. I'd say "quite" was the four, not the "almost not at all".

R: That's not the question. Here, "quite" means more than "not at all". Do you worry about housework, now?

W: No, I shan't be so silly as to get all worked up over that. Give me a naught. That's how we say zero.

R: That means it's not relevant.

...

W: I find doing the dishes four times a day [to be] a bother.

R: I think worry here is supposed to mean stress or anxiety.

W: What?

R: Like, does it get on your nerves?

W: Well then, dear, you understand it better than I. You check what you sees fit.

R: You tell me what you think first.

W: Either you worry or you don't. Some days, it grates on my nerves. Some days it doesn't.

R: Housework?

W: No, that'd be foolish, unless mother's trying to help and its spring cleaning time. I worries that she's too old, but you can't hold her back. Poor dear's had one hard life.

R: (Repeat question and answer items 1 - 4, 0).

W: What did Betty tell you on this one?

R: This one's for you. I need your answer.

W: It's hard to say. I want to do well on this test, but I must be some stunned, because I'd never thought about it like this.

R: It's not a test. In this case, you're the expert. You have all the right answers. I'm just here to learn from you.

W: Well, my dear, if that's how you see it. I'll take the five.

R: There's no five. (Repeat question and answer items 1 - 4, 0).

W: O.K. It's probably a lie, but I'll take the four.

R: Thanks. Ready for the next question?

Such an interaction is not uncommon, although the degree to which the interviewer tolerated questions may be unusual. In the "Results" section of a typical

publication arising from such an interview, however, all information would be lost except that the answer was "Four". Depending on who is asking what kinds of questions, what is important in this interchange could vary from the ultimate answer ("Four"), to the nature of the relationship between interviewer and interviewee, to what the discussion itself indicates about the respondent's feelings about housework. Each of these is a valid question; what I wish to convey here is that the process of asking and answering questions in any research, including medical research, is complex, and that decisions about what questions to ask or which projects fund are not neutral.

Multidisciplinary research is in vogue. The apparent consensus is that tropical diseases, indeed most health conditions, are multifactorial and must be addressed in an integrated way with respect to both research and control strategies.

So, how does this work in practice? *Is there much inter- or transdisciplinary research?* I would argue that there are indeed multiple disciplines involved, but that they are largely working in parallel. The problems and questions considered interesting or important, the methodological approaches considered valid, and the definition of the object of research, are those of the particular discipline of the researcher. They are also partially determined by the history of research on a given subject and the availability of funding.

That there are major -- at times it would seem, irreconcilable -- differences in the theoretical frameworks that guide various research agendas, from the selection and formulation of research questions to the analysis and utilization of data, is no surprise to most social scientists. However, natural scientists in general and biomedical researchers in particular often seem blissfully ignorant of the existence of other, equally valid ways of understanding reality; they seem genuinely unaware of the idea that *their* work is *also* shaped in its every aspect by often implicit assumptions.

This can be critically important when undertaking multidisciplinary research in health, because the combination of the point of view that biomedicine is just studying "objective reality", the political and economic power held by physicians, and the urgency to *do something* that physicians develop almost as a reflex makes it very difficult for other voices to be heard. As a consequence, the questions, priorities and conceptual frameworks brought to bear on the problem of ill health related to endemic diseases are usually determined by biomedicine; "multidisciplinary research" often becomes no more than the piecemeal addition of techniques developed by other disciplines. For example, there has been a good deal of research on schistosomiasis in Mali, but it has *not* been focused on the fact that bilharzia was not endemic to Mali until certain irrigation projects were undertaken (U. Brinkman, personal communication). Thus, research questions that would be of major interest to social scientists, such as the reasons for, nature of, and impact on local populations of so-called development projects are not asked, and social scientists are invited to suggest good ways of persuading the local people to use latrines.

That multidisciplinary research can be much more than this is beautifully shown by some of the papers to be presented at this meeting. Biomedical and social science researchers and the funding agencies involved in endemic disease research need to take an objective step back, look at our assumptions and their implications, and begin in earnest a dynamic, iterative process of narrow focus and analysis, the hallmarks and source of power of the natural sciences, with synthesis and an examination of what happens at other levels and interfaces of reality.

In moving towards cooperation with other researchers and away from cooptation:

***Step One* on the road to creating and sharing knowledge for action is: overcome M.D.-centrism and acknowledge that it is not just the others who have a theoretical framework.**

What is the nature of the theoretical framework guiding biomedical research in endemic diseases? There have been literally volumes written on this question, and the answer is far from being agreed upon. However, the particular aspect that I would like to draw attention to is the prevailing view held by many scientists and large segments of the population: that science is "true", that it is objective and value-free, that the role of the scientist is to discover and document, from a position of objectivity and neutrality, the reality that is "out there". Some corollaries of this are that scientific work is guided by rational choices having to do only with the scientific questions under study, and that it is not really the scientist's responsibility to "use" the knowledge he or she gains.

A different view, whose best known proponent is perhaps Thomas Kuhn through his book "The Structure of Scientific Revolutions", sees sciences as a human endeavour, and therefore inevitably social, cultural, economic and political. Such a view helps to explain some of the apparent "inconsistencies" to be found at every turn.

By way of example, Judith Justice's anthropological studies of primary health care bureaucracies found, among other things, that countries adapted their existing health care structures and manipulated the identity and terminology of programs in order to accommodate the new international directive of primary health care. Rather than encouraging creative, research-based approaches that responded to their own needs, ministries of health re-named erstwhile malaria workers as "village health volunteers". This is perhaps "unscientific", but it is hardly surprising, in Justice's view, given the way that new strategies are promoted by international agencies. A country such as Nepal, about half of whose budget comes from foreign aid, can hardly do otherwise in a setting where, as Justice writes in 1987, "within a decade international policy shifted from

vertical approaches to integrated basic health to community participation to P.H.C.", then back to vertical programs of diarrhoeal disease control and immunization, which formed the core of the "child survival" policy. In 1987, there were indications that the child survival policy was already being challenged by yet another new priority: "safe motherhood" (Justice 1986 & 1987). Funding agencies need to see their respective "priorities" in a historical, political and cultural context, rather than as the ultimate, obvious and scientifically proven "truth".

Uriel Kitron wrote a paper for the 1988 Takemi Symposium, published in "Towards International Co-operation in Health" (Reich and Marui 1989). He discusses an "integrated disease management" approach to tropical diseases, using as a model the "Integrated Pest Management" approach familiar to agricultural and ecological researchers. In analyzing successful and unsuccessful programmes addressing malaria and schistosomiasis, he and Spielman developed a semi-qualitative chart of the relative amounts of various kinds of resources needed for different types of disease management strategies. For example, strategies based on drugs and vaccines need a high level of external resources and at least district or even national levels of structural organization. On the other hand, small scale source reduction depends on community participation, local research, and local maintenance, but is independent of major external funding and only requires organization at the community level. It would seem "rational", given current and foreseeable economic and structural realities, to focus attention on those control strategies least dependent on lots of money and sophisticated organization, but this has not typically been the focus: the "interesting" research questions, especially if you want to advance your career, are at the vaccine and drug end of the spectrum. This, of course, reflects what biomedical researchers are trained in and where the money is: for the researchers, certainly, but perhaps more importantly, for the pharmaceutical and other companies involved. While developing countries may not be an attractive market for expensive drugs and vaccines, the countries producing the research are such a market. The WHO is also not an insignificant purchaser of drugs. That such considerations guide research choices is understandable if science is seen as a human and therefore cultural and political activity, but as long as the implicit and explicit assumption is of objectivity and neutrality, we can only continue to plug away at "interesting questions", provided that donors also think they are interesting.

A historical perspective is essential in order to understand the conceptual frameworks currently guiding both medical and social scientists. A discussion of the history of tropical diseases research and of the historical relationships between medicine, public health and anthropology is beyond the scope of this paper, but colonialism has played a decisive role in each of these fields. In the case of anthropology, the association of ethnographers with the strategic aims of the colonial powers has left both anthropologists and former colonies with bitter legacies. In some countries, such as Senegal, social sciences faculties were closed at independence. The apparent extreme reluctance of anthropologists to do anything that might seem to manipulate local populations on behalf of what might be interpreted as new colonizers -- economic,

political and scientific -- can be seen to stem at least in part from this colonial legacy. On the other hand, the very existence of tropical disease research *per se* in rich countries can be attributed, again at least in part, to this same colonial and military heritage.

Multidisciplinary research seems much less problematic in other fields; oceanography in the 1970's provides an excellent example of researchers from diverse backgrounds working together to understand the sea. One major difference between oceanography and endemic disease research is the absence in the former case of a strong normative element to "save lives and stamp out disease", whose "protector" in turn also happened to be the participating discipline with the most economic and political clout. Again, as long as biomedical research is seen as "value-free", this very real factor cannot even be legitimately discussed, let alone resolved. Discussion is limited to innuendo and grumbling, or explicit critiques to audiences of other "believers" at social science conferences.

In the introduction to "La construction des sciences", a text on the philosophy of science, Gérard Fourez explains that philosophy is a specialized branch of knowledge with its own history, tools, and methods of analysis, and just as it would be absurd to claim to know advanced mathematics without studying calculus, or to understand physics or chemistry without learning their basic concepts, so is it impossible to understand philosophy without studying *its* concepts and methodologies. He adds that it seems silly to have to point this out, except that natural scientists seem to lose all concern with scientific rigour the minute they step out of their own disciplinary boundaries (Fourez 1988). Biomedical researchers need to acknowledge that science itself is ultimately based on philosophical tenets, and to be aware of what some of these tenets are. It follows:

Step Two on the road to creating and sharing knowledge for action is: move away from narrow unidisciplinarity and recognize that different disciplines have strengths and weaknesses, and that your own does not have a monopoly on rigour.

George Foster is a senior professor of anthropology at the University of California at Berkeley. He has a long and distinguished career which has included frequent consultation for the WHO. In 1987, he published a rather blunt but hopefully constructive account of the frustrations of doing behavioural and social science research in the WHO. The main problem, as he saw it, was that doctors think that, by virtue of their training and experience as physicians, they are also qualified and capable of evaluating and doing first class social science research (Foster 1987). While Step Two is applicable to all disciplines, given the previous discussion of agenda-setting and power it is particularly directed at my biomedical colleagues, who are exhorted to repeat it out loud several times a day.

A truism of social sciences is that human beings are *by definition* social; Bonilla Castro and her co-authors write in "Salud y desarrollo - Aspectos socioeconómicos de la malaria en Colombia" that "the social is above all the essence of human nature, and not just a condition of life" (Bonilla Castro et al. 1991). Such a perspective allows, at least in principle, a narrowing of the artificial chasm between the "social or cultural" and the "biological" (or implicitly "real"), and sets the groundwork for a truly integrated approach to understanding health and the human condition in its entirety.

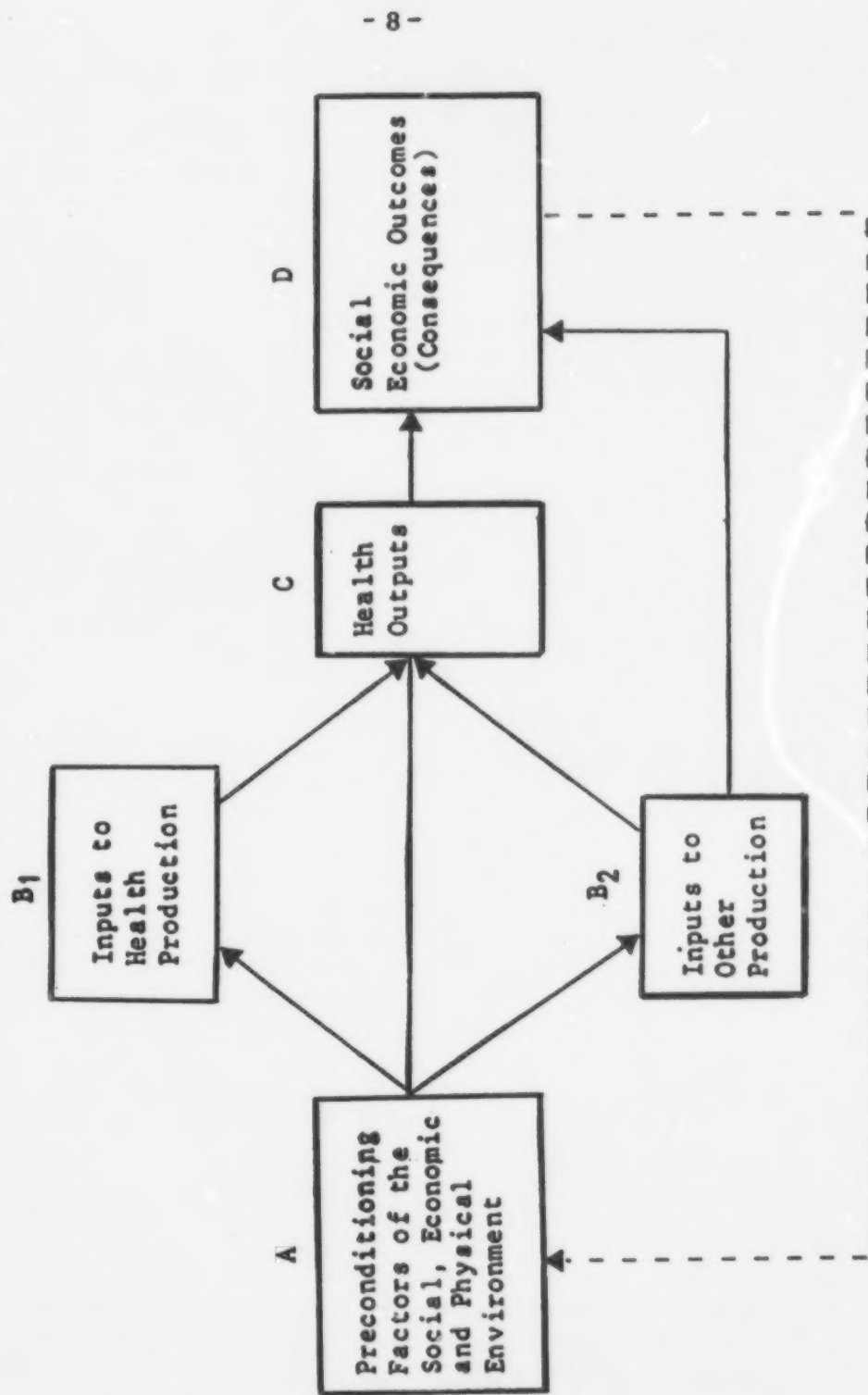
Since understanding and improving the human condition is what all of this is about, it is imperative that each of us continually examine how we are contributing, what our limits are, and where other expertise -- whether local knowledge, other disciplines, or policy-oriented -- is necessary. It is not a straightforward process; the interview which is quoted above illustrates how messy this attempt to understand complex human phenomena can be. Nevertheless, it is not permissible to hide in a statistics book with the excuse that this is too uncontrolled, or that qualitative data are a nightmare to analyze. Indeed they can be, but just as most biostatistics students soon get over their naive hope to immediately and intuitively understand varimax rotations of factor analysis, so we can take a deep breath and prepare to learn at least the right questions to ask in other fields of knowledge.

We've come a long way. Following are two diagrams from TDR-SER publications, ten years apart (WHO 1980; WHO 1991). The first shows a diagram from the "suggested" conceptual framework for research on the social and economic impact of tropical diseases. As you can see, it is quite narrowly economic, and while it is still reflective of one school of social sciences -- a particular type of economic analysis -- it is certainly not the only nor even perhaps the most appropriate conceptual framework for social science research about tropical diseases.

The second diagram comes from a meeting held earlier this year on the application of rapid assessment methods to tropical diseases. Although it continues to use some categories relevant especially to the "default" disciplines (medical science and epidemiology), the approach is much more global and allows for analysis of different levels of reality. The key column here is the last one: PURPOSE, which could have many more entries. If the purpose is to document incidence rates of cutaneous leishmaniasis among miners in the jungle, different approaches are necessary than if the purpose is to develop valid indicators of social conflict arising from differential access to treatment.

Finally, having begun an examination of the theoretical and ideological assumptions underlying the natural sciences and biomedicine in particular, and having accepted that no discipline has all the answers or even most of the questions, we may be ready for:

Figure 1. Conceptual framework for assessing the social and economic consequences of the tropical diseases.



(Source: WHO 1980)

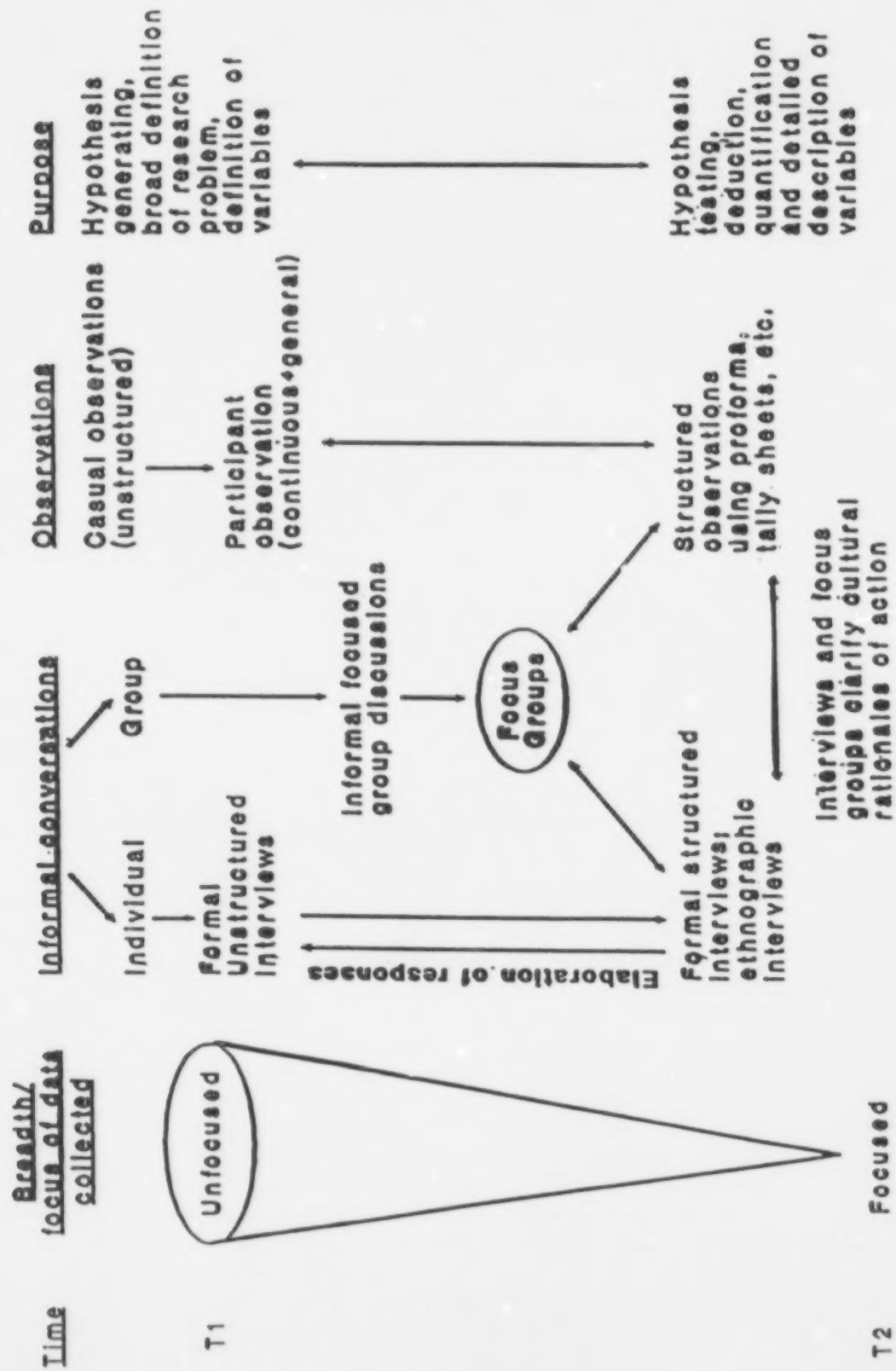


Figure 1. INTEGRATING RESEARCH METHODS (Source: WHO 1991)

Step Three on the road to creating and sharing knowledge for action: resign yourself to long discussion and negotiations with co-researchers, funding agencies, and the policy makers you ultimately hope to influence, and, most importantly, with the people on whose behalf you are doing all this work.

There are no easy answers. There are no fool-proof recipes. There are no magic bullets. One of the books I read to prepare for this talk is called "Biomedical Science and the Third World: Under the Volcano" (Bloom and Cerami 1989). I opened it, expecting a hard-hitting, polemical critique of the broken promises of biomedicine; I found that "Under the Volcano" implied *not* that time is running out, but that the answers to all kinds of problems are just bursting to come forward, to explode in a lava flow of science applied to ending human misery. The individual papers in fact addressed the issue from many perspectives, ranging from the immunology chapters with virtually no vowels to rather depressing economic analyses. Indeed, almost all of the papers were a good deal more cautious than the one generating the title, yet the overall optimism remains. It is one thing to understand that "multidisciplinarity" will likely entail not getting your own way much of the time, but the process of trying to see problems from different points of view and in a broader context than the germ theory of disease, for instance, allows is ongoing and difficult. The temptation to see the latest scientific advance as "The Answer" is extremely powerful.

Although the central purpose of this paper has been to challenge a simplistically linear view of the nature of scientific progress and to avoid replacing it with another simplistic view of "multidisciplinarity" as the new panacea, I would like to hold fiercely to the commitment and hope that underlie that title, beneath the complacency and the facile slogans. It is this commitment of medical scientists, social scientists, communities, and others that may provide the base from which to continue the discussions, negotiations, failures, and successes.

In his collection of essays *La otra voz: poesía y fin de siglo*, Octavio Paz (1990) discusses the threat that blind market forces pose to intimate human activity, such as poetry. He writes:

Today the arts and literature are exposed to a distinct danger: they are not threatened by a doctrine or by an omniscient political party, but by an economic process that is faceless, soulless and without direction. The market is circular, impersonal, impartial and inflexible. Some will say that, in its own way, it is just. Perhaps. But it is blind and deaf, it does not love literature or risk, it does not know and cannot choose. Its censure is not ideological; it has no ideas. It knows of prices, not of values. (my translation)

Science is also impartial and inflexible; science also has no ideas. But science as a human endeavour *is* ideas, and it is up to us to create and share those ideas, to imbue them with human values of justice and compassion. To do otherwise is to betray its promise, and ours.

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Ecological and Environmental (Eco-Epidemiological) Approaches to Leishmaniasis Control

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In the various scientific meetings at which we meet from time to time, and in the hundreds of publications which appear every year on the subject, we have come to appreciate the extraordinary variety and complexity of the group of diseases known as leishmaniasis.

The complexity of this subject makes its study an exciting challenge. However, in those countries where leishmaniasis is both a problem of public health and also sometimes of public order, studying it leads to a commitment to those who suffer from it: this colours the research, from its very inception, in terms of the struggle against the disease.

Research can be focused in many ways. Allow me to present today the eco-epidemiological analytical method, which has already been elevated in some quarters to the status of a new science, eco-epidemiology, the offspring of the union of ecology and epidemiology.

Eco-epidemiology begins with the notion of the "natural locus of infection" developed by Pavlovski (1964), which shows that the spatial distribution of any animal or vegetable species is determined by specific ecological conditions, among which climate plays a predominant role.

Rioux and colleagues of Montpellier, France (Rioux et al. 1968; 1973; 1981; 1986) have applied Pavlovski's concepts to the study of leishmaniasis, taking into account the fact that the transmission of this zoonosis within a natural locus of infection depends essentially on the presence of the three elements of the cycle: parasite, vector and reservoir. In turn, the presence of these elements is conditioned by such factors as climate, flora, fauna, soil composition and geomorphology.

This analytical method is based upon a global (holistic) understanding of how the locus of infection functions. It requires an interdisciplinary effort in which the various disciplines each bring their specific tools to bear on the common objective. The results derived from the application of these disciplines are then brought together, inter-related and structurally related, in order to determine the particular influence each of them exerts on the presence and persistence of the locus of infection.

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As will be shown below, this method allows the weakest points in the epidemiological chain to be identified, and simple and realistic control measures to be formulated that are commensurate with the resources of the countries and populations we are trying to protect. It is important that such strategies be environmentally friendly and oriented toward an overall improvement in the living conditions of the population.

Although a general methodology is available, there are no "recipes" for studying the locus. The eco-epidemiological method is above all an approach which utilizes complex techniques to take into account the natural history of a disease and its multiple causes. The concepts and techniques of epidemiology are used to study the distribution of a disease and the determinants of its frequency in an area. The tools of ecology are used to analyse and compare the communities (biocenosis), the ecosystems and the impact of the changes to the biosphere wrought by man (noosphere) in those places where transmission occurs and in those where it does not. In this way it is possible, for example, to relate the presence and the density of the vector or of the reservoir to a bio- or phytoclimatic zone and to develop ecological markers and nosological maps of the macro-, meso- and micro-loci.

The methodology in general begins with the localization of the zone in which the disease is endemic; this is achieved by establishing the places of contagion of known cases, which act as markers for this purpose. The study of the region can be carried out exhaustively with techniques such as the "cartographic net" used by Rioux (1986) in Corsica and by Lannote (1974) in the south of France. This technique requires more intensive sampling and commensurately greater resources. The line transect technique, widely used in ecology, allows the taking of homogeneous samples at different levels (of spatial location, altitude or climate) of the various elements which intervene directly or indirectly in the transmission of the disease. The transect must not be restricted to the interior of the locus, but must cut across it, along various axes, in order to make the differences observable.

Once the places of contagion have been established, the multidisciplinary study of the structure and functioning of the locus of infection can begin. The techniques and disciplines involved vary as a function of the particular characteristics of the area of study. The elements are thus available to permit the determination of the epidemiological risk and finally to identify the key points where intervention is necessary to establish control.

For instance, in 1960 Rioux designed a study of leishmaniasis in the south of France using two complementary orientations: the ecology of the Phlebotomus on the one hand, and the ecology of the wild and domestic reservoirs on the other. The zone of study was part of the Mediterranean sub-region, with a Mediterranean climate. The origin of the cases showed an inhomogeneous distribution within the Mediterranean sub-region.

In order to observe the differences, Emberger's pluviothermic coefficient was used to differentiate six meso-climates, corresponding to six different bio-climatic zones: Saharan, Arid, Semi-arid, Sub-humid, Humid and High Mountain. Each bio-climatic zone contains a characteristic class of vegetation (vegetation base), constituting the unit of reference for the analysis. The study of the distribution of Phlebotomus was done using the transect technique, using paper traps impregnated with castor oil.

It was shown that Plebotomus ariasi was the vector species. It was found over the range of 100 to 1300 metres above mean sea level (with greatest density between 300 and 450 metres), in the vegetation base populated simultaneously by two types of oak: Quercus ilex and Quercus pubescens. The simultaneous presence of these two types of tree indicated the risk zone (ecological markers).

In 1975 Lanotte conducted a canine survey, using indirect immunofluorescence, and once again an unequal distribution of the infection was observed in the canine reservoir. By correlating the frequency of canine leishmaniasis and the density of Plebotomus ariasi, the minimum relative density of the vector leading to transmission was determined. This information is very important since it allows a more precise delimitation of the risk zone.

The adult vector P. ariasi is active during the hot months (June to September). Determining the physiological age and the gestation index of females throughout this period, Guilvard et al. (1980) found that the greatest density of multiparous infected females occurs at the beginning of the autumn, so that this is the transmission period. In all isolations, the iso-enzymatic identification of the stocks showed that Leishmania infantum was the causal agent of human (both visceral and cutaneous), canine, murine and vulpine leishmaniasis.

At present, there are very few cases of human leishmaniasis in France, and both people and dogs have easy access to consultation and treatment. The control measures which have been recommended are oriented to avoiding contact between the canine population and the infected vector, given the epidemiological risk.

The value of the eco-epidemiological method has also been seen in a completely different region, namely the province of Tata, in southern Morocco. In this zone, the Moroccan health authorities advised of the sporadic occurrence of human cases of Aleppo ulcer. Beginning in 1977, there were epidemic outbreaks affecting thousands of people. Similar phenomena occurred at about the same time in Libya, Tunisia and Algeria.

Field studies made it possible to determine the clinical characteristics of the disease, which was restricted to the oases located along the "rivers" (wedi) of the south of the Anti Atlas. The Montenegro Test, applied to 8473 persons, showed the

population's early contact with the parasite, the epidemic character of the outbreak and a difference in the frequency of infection in the various social strata of the population.

Through isoenzymes identified by electrophoresis, the responsible parasite was found to be Leishmania major Mon 25. Earlier studies by Soviet researchers showed that cutaneous leishmaniasis caused by L. major is a zoonosis and that its reservoirs are small mammals of the family gerbillidae.

In this study, Meriones shawi grandis was found to be naturally infected, with chronic lesions in the auricular pavilion, and it was shown to be the reservoir in this zone. This rodent lives in the oasis, close to dwellings and in cultivated fields, since it needs free-flowing water to survive; it feeds on plants, but also on garbage and human excrement, and follows humans to new settlements.

Correspondence analysis of the species of Phlebotomus captured at each station and the ecological profiles showed that P. papatasi and P. alexandri were found preferentially in the arid and peri-arid zones. The isolation of L. major in P. papatasi confirmed the incrimination of this species as the vector in the arid and peri-arid areas, which are the risk zones. Fringe shanty towns are generally populated by low-income families; near these areas, people dig up earth to build their dwellings, creating hollows which later become garbage dumps and public latrines. The greatest density of vectors and infected reservoirs is found in these hollows.

At the end of the summer, when the female vectors have their highest reproductive rates, and because of the temperature variations peculiar to the Saharan climate, P. papatasi seeks refuge inside dwellings and bites people.

Hence from this information it is possible to differentiate the macro-locus, the hyper-arid areas where the vector P. papatasi is found; the meso-locus, made up of the oases (palm trees and inhabited housing); and the micro-loci, corresponding to the garbage dumps where vectors and reservoirs live out their life-cycle and sustain the parasite.

The appearance of epidemic outbreaks is the result of the presence of multiple factors. Torrential rainfall allows the growth of a large quantity of plants which serve as food for the reservoir, and this leads to a great increase in the population. At the end of the summer, following the maximum peak density of the vector, the greatest transmission occurs, both for the reservoir (epizootic) and for humans (epidemic) (Rioux et al. 1990). The population at risk is made up of people who have recently moved to the area, children born between epidemics and particularly the poorest people who live in the peripheral shanty towns where hygienic conditions are most precarious.

Conclusion

The above provides some examples of studies of loci which follow the eco-epidemiological model. However, it is important to keep in mind that successfully combatting such complex diseases as leishmaniasis requires the careful design of control programs and strategies.

The objective of control programs is to reduce the incidence of the disease so that it ceases to be a public health problem. These are programs of indefinite duration which are implemented only in those areas with greatest epidemiological risk, and they must be designed so that program activities may be integrated with the duties of available personnel, within the framework of their regular work.

But let us be realistic. The control must be integrated, acting on the weakest and most accessible points of the epidemiological chain, and to be effective there must have a large community participation component.

In our experience in Colombia, community participation has been decisive in the study of loci of cutaneous leishmaniasis. Given motivation and basic training, the community itself participates in the identification of specific entomological markers and in the search for cases. Community participation will be assured as long as we know how to get people involved and see that they undertake the control program as a matter of community interest.

Let us not forget, when designing control measures, that in under-developed countries the feasibility of a program must be judged more in terms of cost than in terms of efficiency. A control program must be designed in light of the resources available and specific local conditions and in all cases the epidemiological risk must be taken into account. With the application of the eco-epidemiological method, we gain a better understanding of the transmission process, and are better able to identify the zones, periods, locations and population at greatest risk of infection.

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A Strategy For Control Of Cutaneous Leishmaniasis Through The CIMDER's Primary Health Care Model

Jaime Becerra¹ and Marleny Muñoz²

Introduction

Three years ago Kenneth S. Warren, honoured by the London School of Hygiene and Tropical Medicine with the Heath Clark Lecture, brought into discussion the old conflict between tropical medicine and tropical health, between scientific research and public health. The antagonism between Sir Patrick Manson and Sir Ronald Ross served as the historical reference to dissect the evolution of the two fields. Although both men were clear about the goal of "science for development" differences appeared when considering the means to this end. Manson's interest in the strength of biomedicine represented scientific research for development, while Ross's concern with operation of results acted in the name of public health for development. Warren's main conclusion seems to be a good reference point to keep in mind during our paper: **"Today, the conflict between tropical medicine and tropical health is being resolved with the realization that they are truly complementary disciplines"** (Warren 1990).

The inherent complexity of tropical diseases is fertile ground for allowing the settlement of multiple disciplines. The study of diseases occurring within the zone lying between the Tropics of Cancer and Capricorn, has been a multidisciplinary effort. In 1975, when the World Health Organization (WHO) initiated its Tropical Diseases Research Program (TDR), a new age of tropical medicine was established. Since then, a large amount of money has been allocated by institutions like the Rockefeller Foundation, the International Development Research Center (IDRC), WHO, the MacArthur Foundation, and others, to the study of the biomedical aspects of parasitic diseases. The results have been striking, especially in the fields of molecular biology, biochemical pharmacology and parasitology, to the understanding of host-parasite relationships, the refinement of diagnostic tools and the path towards vaccine development (Warren 1990).

An important proportion of funds has also been devoted to the inquiry of priorities in terms of sociology and economics (UNDP/World Bank/WHO-TDR 1980; Rosenfield, Goladay, Davison 1984). Today, there is a better understanding of factors neglected in the past: the behaviour of man in the cycles of transmission, mobility and migration as a key factor in the perpetuation of disease, and the microfocality nature of transmission, among others (Gillet 1985; Mata 1982; Sevilla et al. 1988; Bonilla et al. 1991).

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The interdisciplinary approach has been tried at least with some of the tropical diseases (UNDP/World Bank/TDR 1980). It is clear that when a science requires information coming from another science to solve problems in such a way that an interaction to transform the science is established, the interdisciplinary approach is real. In this sense, the impact of interdisciplinary teams working directly towards the control of tropical diseases remains to be evaluated.

The model we propose at this meeting is the result of a joint effort between two Colombian research institutions to move forward in the study of tropical diseases. The International Center for Medical Training and Research (CIDEIM), and the Center for Multidisciplinary Research on Development (CIMDER), are working together in order to combine the experience of the two institutions to develop a strategy based on social participation to control Cutaneous Leishmaniasis in the Colombian Pacific Coast. This initiative is an encouraging example of interdisciplinary cooperation for research and research for development. CIDEIM is a center with broad biomedical experience, having explored for more than a decade the clinical, parasitological, immunological and epidemiological aspects of leishmaniasis (Weigle and Saravia 1990). As a complement to this, for more than 15 years CIMDER has been involved in health services research and development. CIMDER has designed, tested and evaluated Primary Health Care (PHC) strategies, technologies, and training models which have been used at the local, national, and international levels (Salazar 1991).

Our hypothesis is that through the implementation of a PHC strategy currently operating in Colombia, adjusted to the particular reality and characteristics of cutaneous leishmaniasis, we will be able to develop a model for control suitable not only to leishmaniasis but to other tropical diseases as well. Using Kenneth Warren's words: We have realized that we had been applying complementary approaches waiting to be merged as a synergistic tool.

CIMDER's Primary Health Care Model

CIMDER is a non-governmental organization (NGO) nested within the Universidad del Valle, the third major official university in Colombia. Our group started as a multidisciplinary team in 1974. The changing realities, needs and resources at the community and academic levels compelled us to shift towards an interdisciplinary approach to research, although we hope in the future to evolve to a transdisciplinary stage in which a total system with no definite frontiers between disciplines, no definite frontiers between communities and researchers, is possible.

Since the founding of the center we have been devoted to Operative Research, defining our mission as: **the design of strategies to increase wellbeing of communities taking the health sector as the starting point.** Years before the Alma Ata Conference, we were working to approach development based on many of the principles later covered by the PHC concept (WHO 1978). From our perspective PHC is defined as the local

and permanent production of individual, collective, and institutional answers to the health problems. The model we have created is aimed at the development and strengthening of this production ability, focusing on three sectors as the target of actions: Community, Municipal Administration, and Health Institutions (Figure 1).

Every sector has been assumed as an avenue of research to carry out studies concentrated on the design and testing of methods and technology to improve the model. Following, we will summarize some characteristic components of this model with special emphasis on the community sector.

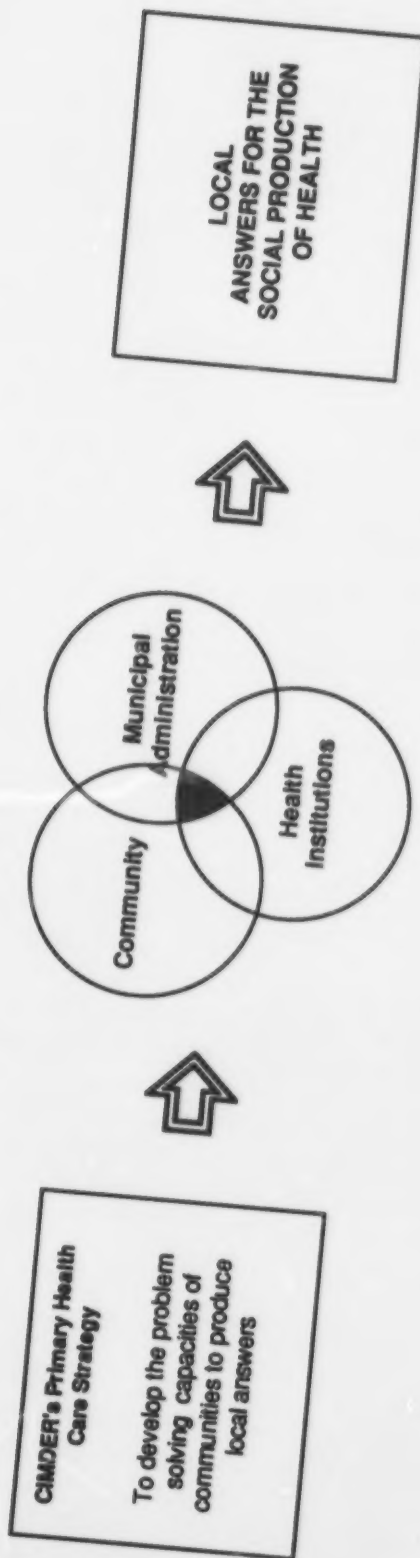
At the community level, work has focused on family leaders, community health workers (health promoters), and health volunteers. In the case of family leaders, we consider the mother as the center of actions. We have designed a participant pedagogy strategy called **"School for Mothers"**, with the following objectives:

- i. To increase the mother's problem solving capacity in PHC.
- ii. To promote self-care in health.
- iii. To favour creation and strengthening of groups, community participation, and municipal development.
- iv. To identify health problems, exposed people and priority risks.
- v. To transform the home into an integral part of the first level of health attention.

The educational methodology created to cover these objectives is a non-formal education program and a technological package produced using participatory criteria (Salazar and Becerra 1989). The final product is a transformed woman looking at herself in the mirror of self-esteem and determination. The instruments used to implement "School for Mothers" are intended to produce an act of appropriation by the mother. The *Health Educator Handbook* may be used by the mother to empower other mothers via a mother-to-mother teaching process. The handbook *Let's Talk About Our Health: Solutions to Prevent and Cure at Home*, is a practical and easy-to-use manual written in a colloquial style to implement risk focused health education at the household level. The *Health Advisor* is a sort of health file to give a quick help to solve specific health problems. The *Risk Identification Card* is a tool to help mothers identify risk factors existing at home and in the community. Finally, a Family Learning Network, a Knowledge Transmission Network, and a Solidarity Network are achieved.

Currently, there are "Schools for Mothers" in nine Colombian states, and during the last two years 1,134 mothers have been educated through this program. "School for Mothers" has been adopted by UNICEF in Colombia in order to strengthen the 55,461

Figure 1
CIMDER's Primary Health Care Model



Welfare Homes with community mothers who are supported by the Colombian Family Welfare Institute and take care of 831,915 children.

Working with Health Promoters, who are community health workers paid by the state, is another front of community action pursued by the CIMDER PHC model with the following objectives (Salazar 1990):

- i. To endow the Health Promoter with methodologies and instruments to help them to effectively perform their functions.
- ii. To generate a community health worker profile appropriate to community needs, historical conditions, and local reality.

To reach these objectives the Health Promoters have a technological package with the following tools: A community-based Health Information System, a Micro-Health Post, a tri-coloured strip to assess children's nutritional status, a modified Snellen Table, and a multi-pocket bag. Approximately 20% of the Health Promoters in Colombia have been trained with this methodology.

The Health Volunteers are members of the community, non-paid, and usually women, who represent another effort to integrate people, non-formal education, community participation, and the social production of health. The objectives of this work are:

- i. To strengthen the capacity of the health volunteer to use methodologies and instruments to generate answers to health problems at the local level.
- ii. To promote a process of improvement of living conditions.
- iii. To encourage the use of simple and uncomplex instruments to plan (diagnose, program, execute, monitor, and evaluate) health actions.
- iv. To transform health volunteers into active subjects and not collaborative objects.
- v. To establish a mechanism to increase coverage in the health sector.

At present, around 7,680 health volunteers are deployed within 13 Colombian State Health Services. At the municipal level, our work has been directed to the Community Participation Committee, a governmental instrument created to regulate community participation. The objectives of this work are:

- i. To design Strategic Planning tools to be used in the health municipalization process. This way, the power of communities to co-manage health with state and local directives, must be strengthened.

- ii. To generate methods, instruments and mechanisms to process information at the municipal level to establish an input to the decision taking process.
- iii. To fortify the communication process between the health sector and different community groups.

At the institutional level work has been focusing on increasing efficiency, technological development, and the generation of a culture of dialogue between the service givers and takers at local and university hospitals. Software packages and workshops have been developed to help at crucial points in the hospital administration: supplies, personnel, finance, costs, information, systems, leadership.

A Model for Control of Cutaneous Leishmaniasis

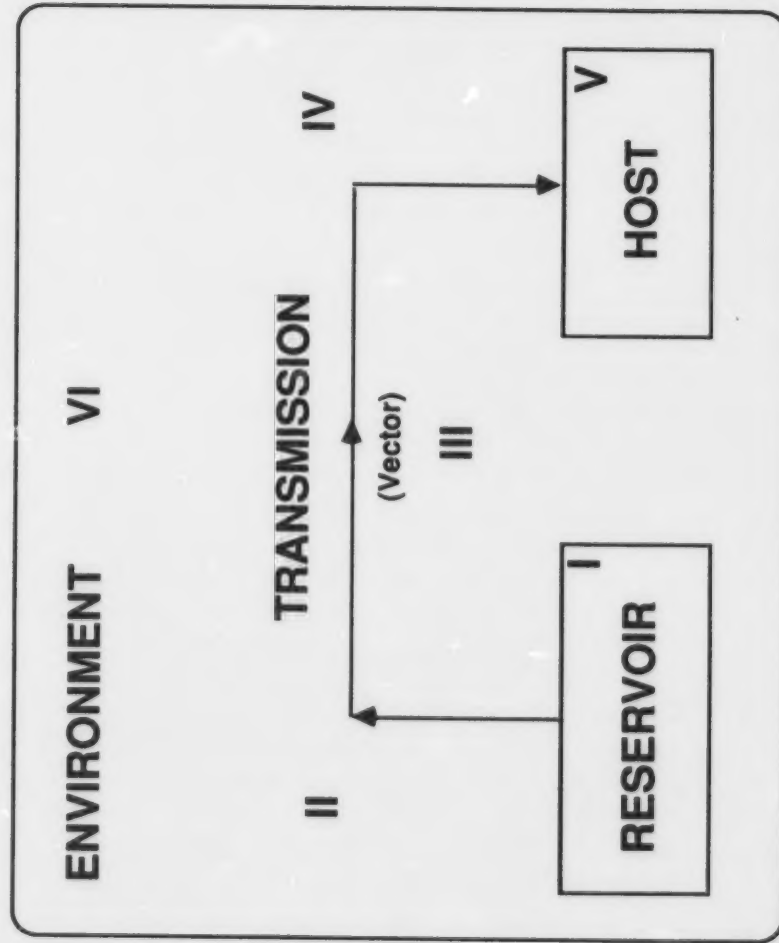
Our starting point is a theoretical model for control of infectious diseases developed by Alzate at the Universidad del Valle (Alzate 1987). This model has been applied to the training of medical and postgraduate students in public health and epidemiology, and to cost-benefit appraisal of public health interventions. Four elements are considered in the model: reservoir, transmission, host, and environment (Figure 2).

We consider a reservoir to be any person, animal, arthropod, plant, soil or substance (or combination of these) in which an infectious agent normally lives and multiplies, on which it depends primarily for survival, and where it reproduces itself in such manner that it can be transmitted to a susceptible host. The transmission is any mechanism by which an infectious agent is spread from a source or reservoir to a person (direct, indirect, and airborne). The host is a person or other living animal, including birds and arthropods, that affords subsistence or lodgement to an infectious agent under natural conditions (Benenson 1985). The environment refers to the physical and social conditions around transmission.

Six strategies for control are derived from these elements:

- I. To keep the size of the reservoir to the minimum.
- II. To avoid the entrance of the infectious agent into the environment.
- III. To interrupt transmission.
- IV. To avoid the entrance of the agent into the new host.
- V. To improve the resistance of the new host.
- VI. To modify the whole environment.

Figure 2
A Model for Control of Infectious Diseases



(Modified from Alzate, A., 1987)

It is not our aim to oversimplify the extremely diverse array of disease manifestations produced by the different leishmania (Walton, Wijeyaratne, and Modabber 1987). However, for brevity's sake, we will restrict our proposal to cutaneous leishmaniasis keeping in mind the similarity in the transmission cycle for the group of diseases that constitute the leishmaniasis.

A strategic plan combining control measures dependant upon the epidemiological characteristics of the foci, has been advocated in the past (Marsden 1984). Insecticide spraying to attack the vector, destruction of animal reservoirs, and the treatment of infected people have given some successful results in few countries. However, the fact that leishmaniasis is a neglected disease, ignorance about insect vectors and animal reservoirs especially in forest leishmaniasis, and the lack of a good pharmacological support against the disease, presents obstacles for implementing these measures in many cases.

PHC as an option to integrate leishmaniasis control has been reviewed (WHO 1990; De Raadt 1987). Some of the advantages are that: (i) wider geographic coverage of control activities can be obtained; (ii) continuity of activities is enhanced since the responsibility for implementation rests in the hands of the population affected; and (iii) the cost of the strategy is low.

Figure 3 presents the proposed model for the control of Leishmaniasis. In general terms, it could be considered a "package deal" to interest the community in the whole problem of the social production of health. Three synergistic strategies ("Schools for Mothers" implementation; Community Participation Committees functioning; and Health Institutions support) would be directed mainly towards two points in the disease cycle: transmission and host. The lack of information about the reservoir and some ecological and socioeconomic constraints to environmental modification do not limit the pursuit of these aspects, at least currently.

The "Schools for Mothers" process which seeks to change attitudes and behaviours through knowledge appropriation, gives rise to real community participation in the choice of technological options for leishmaniasis control (Figure 4). The decision to use insecticide-treated bed nets, repellent soap, insecticide spraying, or protective clothing, is taken by the people. Researchers and PHC agents play roles as catalysts who offer appealing possibilities, options and suggestions, not impositions.

The recent cholera outbreak provided a good opportunity to test a partial application of this model. Since June 1990, community work was started with a "School for Mothers" at the Lower Calima, a region near the Colombian Pacific Coast. A year later, when the cholera epidemic arrived from Peru, the 2,784 inhabitants of the zone were organized, prepared and in good condition to face the problem. The net result was a cholera rate around 7 per 10,000 contrasting with the 1-3% at the surrounding area (Collazos and Gutiérrez 1991).

Figure 3

A Model for Control of Leishmaniasis with CIMDER's Strategy

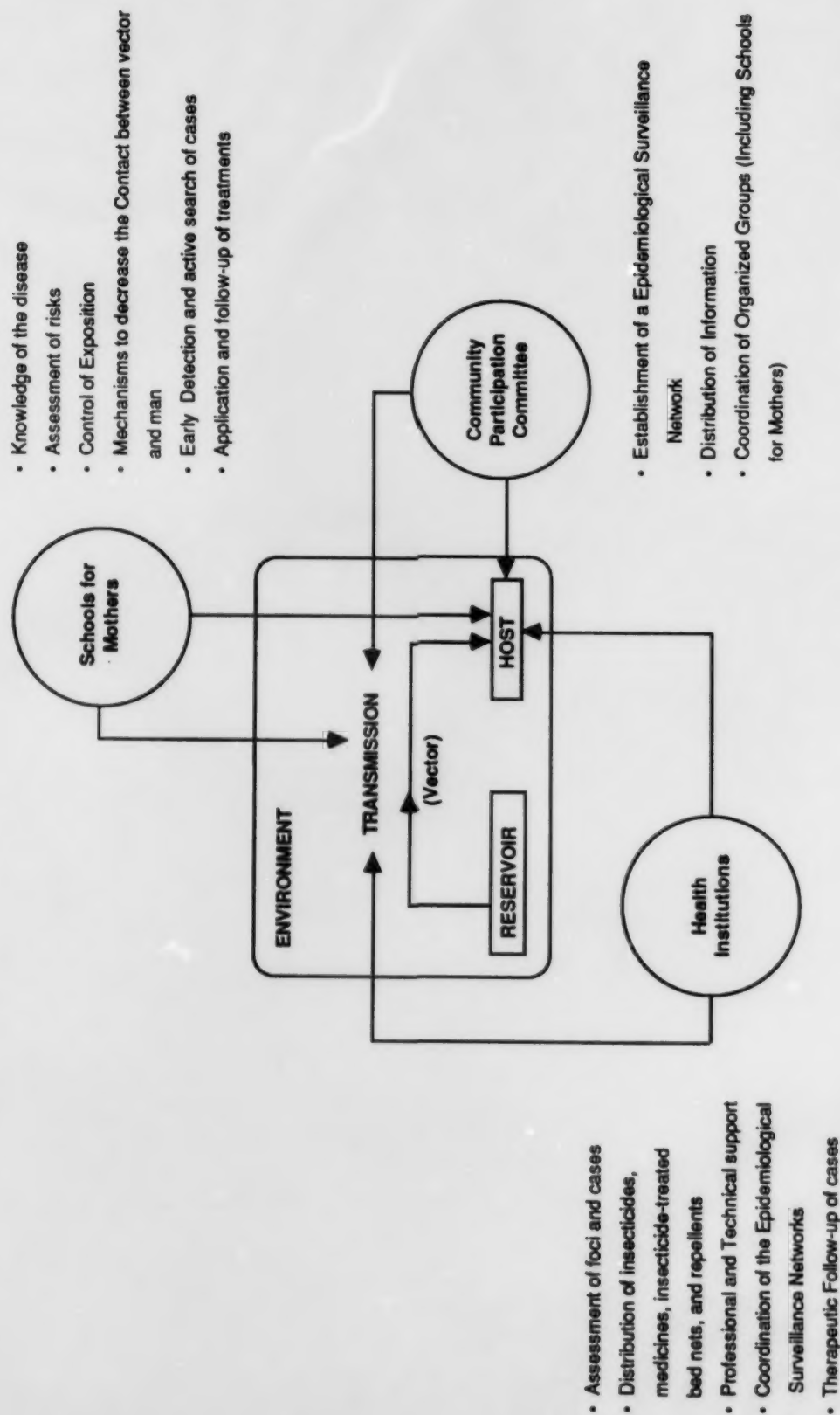
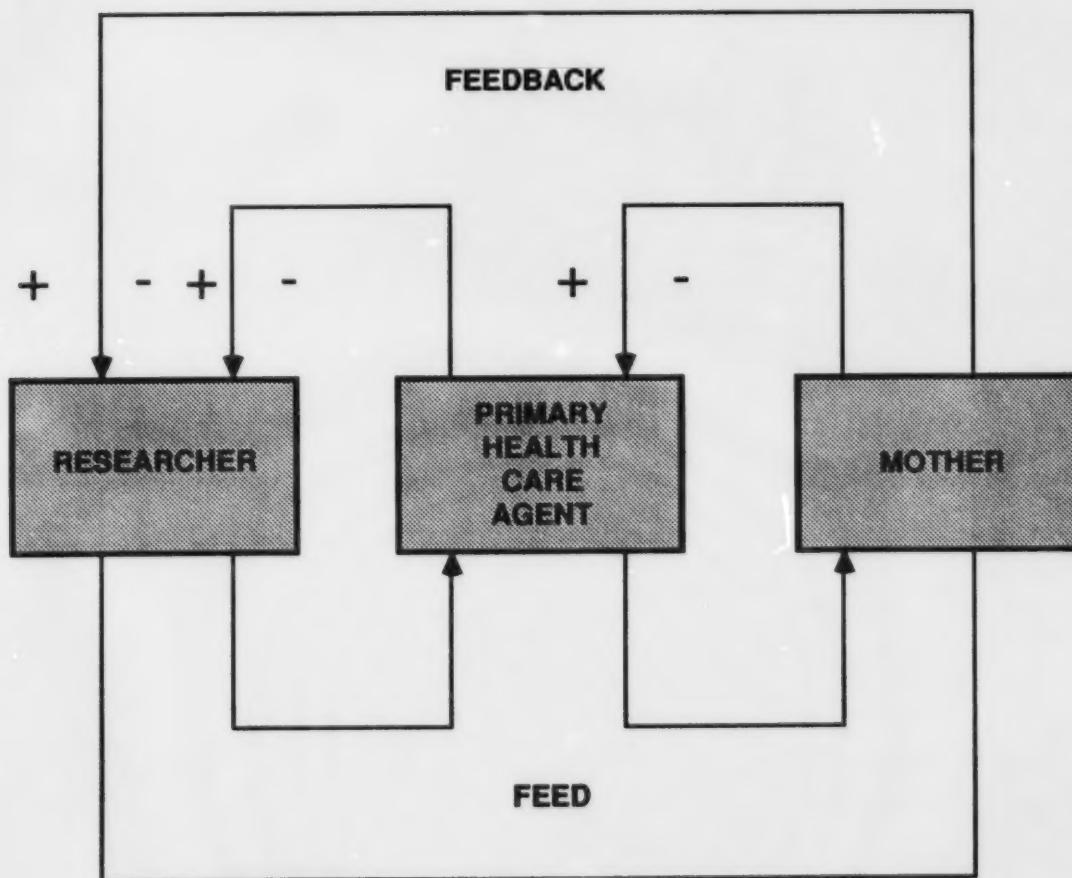


Figure 4
Community Participation in the Choice
of Technological Options in Leishmaniasis Control



Conclusion

Since 1978, when the Alma Ata conference outlined the principles of equitable distribution, community involvement, focus on prevention, appropriate technology, and multisectoral approach to define health for all by the year 2000 as a goal, the disease-targeted approach to control tropical diseases shifted to primary health care (Warren 1990). The political decision taken by more than a hundred countries to obtain a **"level of health that will permit the people to lead a socially and economically productive life"**, established a golden age for primary health care. The option for a selective or a comprehensive approach was later developed (Walsh and Warren 1979). However, even when choosing selective primary health care as the initial strategy to control, its nature of transiency was clear. When regions, countries and programs are asked for results, and this includes tropical health, it is realized that we are at some point on the way to health for all, far away from the beginning but also far away from the goal (WHO 1990).

Recently it has been noted that the conflicting viewpoints polarizing the health policy debate (i.e. selective versus comprehensive primary health care, integrated versus vertical programs, preventive versus curative services) is a confounder because the options are limited to actions that can be carried out by ministries of health (Jamison and Mosley 1991). There is an urgent need to gather, analyze, and concentrate all the available information coming from different fields accepting that they are all pieces of the puzzle. What we need, is a pragmatic approach to the problem of tropical disease control to develop a versatile model to apply at the local level. This model must consider health as a social product and not merely the result of a single sector action. Within this conceptual framework the goal should be the strengthening of the resolute ability of every community to produce answers against tropical diseases.

Acknowledgements

We wish to thank the international institutions that have given financial support to CIMDER during the past 17 years of research activity. The Rockefeller Foundation, the Ford Foundation, WHO/PAHO, UNICEF, Save the Children Federation, Foster Parents International, the German Mission, the London School of Hygiene and Tropical Medicine, the Colombian-Dutch Agreement, and especially the International Development Research Center (IDRC) of Canada.

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Health Policy for Leishmaniasis Control: The Experience of Peru

Marcela Rodríguez¹

Introduction

Human leishmaniasis has long been known to be endemic to Peru. Ceramics from the Nazca culture, in the pre-Inca times, have been found depicting the disease in its most typical form, with mutilating lesions on face, nose and lips. This disease takes two clinical forms in Peru: (i) Andean cutaneous leishmaniasis or "uta" and (ii) jungle cutaneous or mucocutaneous leishmaniasis called "espundia".

Andean Leishmaniasis occurs in the Andean valleys of the west side of the Andes range. Its clinical presentation involves the skin, mainly exposed areas like the face and hands. It rarely presents mucosal involvement. This type of leishmaniasis is usually benign and untreated lesions heal spontaneously. The population affected is generally children and young adults.

Jungle leishmaniasis occurs in the eastern areas of the Andes range, in the Amazon region, and in specific sites of some Andean valleys. The disease produces cutaneous lesions, with a long latent period, usually located on the lower limbs or trunk. With undetermined frequency it involves, by metastasis, the respiratory and oropharyngeal mucosa. It is commonly a disease of young adults, especially if they are recent immigrants.

In Peru, leishmaniasis was officially declared an "occupational disease" in 1975, due to its association with the opening of roads, oil extraction, lumbering, gold mining, and other similar activities. However, given the complexity of the disease, this recognition has not provided any substantial benefit for affected workers.

Later, in 1985, leishmaniasis was included among the Special National Programs for the Control of Metaxenic Diseases, which at that time only included malaria and yellow fever. Despite this, a National Plan for the Control and Prevention of this health threat has not been defined to date.

Some statistical information on the disease has been collected by the Peruvian Ministry of Health. Although incomplete and based on case reporting by passive detection and clinical diagnosis carried out by the health care centers of the Ministry of Health, this data is used in this report to analyze the geographical distribution, prevalence and epidemiological characteristics of leishmaniasis in Peru.

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General Epidemiological Aspects of Leishmaniasis in Peru

The concurrence of ecological factors (i.e. the interaction among the leishmaniasis etiological agent, vectors, and reservoirs) and social and economical factors (which are probably more important in the accidental contact of man within the natural cycle of disease transmission) determines the epidemiological patterns and the magnitude of the occurrence of leishmaniasis in Peru.

A concrete example of this situation occurs in the south Peruvian Andes, where leishmaniasis is related to the temporary migratory movements of the Andean people to natural transmission sites. These are places where people participate in gold mining, lumbering and picking chestnuts. Drug trafficking and terrorism are also important reasons for population migration, exposing individuals to the risk of acquiring leishmaniasis.

The parasite species causing leishmaniasis in Peru are:

- Leishmania (V.) peruviana, causative agent of "uta" or Andean cutaneous leishmaniasis.
- Leishmania (V.) braziliensis, causative agent of jungle cutaneous and mucocutaneous leishmaniasis, also known as "espundia".
- Leishmania (V.) guyanensis, recently isolated from the cutaneous ulcer of a patient coming from the jungle.
- Leishmania mexicana amazonensis, recently isolated from a female patient with non-ulcerative cutaneous lesions that appeared as plaques and multiple nodules over her face, neck, arms, legs and feet, who acquired the disease in the Department of Junin.

Regarding the vectors, Lutzomyia peruensis has been described as the vector of Andean cutaneous leishmaniasis or "uta". The vectors for jungle leishmaniasis remain unknown to date.

As for natural reservoirs, although dogs and some small wild rodents have been found to be naturally infected, their role in the transmission cycle of the disease is still uncertain. The reservoirs involved in the transmission cycle of jungle leishmaniasis have not been defined thus far.

National Reporting System

The National System of Epidemiological Surveillance considers leishmaniasis a communicable disease requiring mandatory reporting, subject to weekly notification.

National statistics record cases as far back as 1951. These are reported by age groups and areas where the case was first detected.

The national system accepts reports of communicable diseases from three levels. Each level has the following functions and individuals in charge:

- (1) The local level, where cases are detected and reported, involves hospitals, health centers, sanitary posts, clinics of various health care entities, research institutes, universities, laboratories, community, etc.
- (2) The regional level, made up by the Regional and Sub-Regional Health Units, and;
- (3) The national level, which is the governing body, represented by the General Office of Epidemiology, the Technical Direction of Data and Statistics, National Control Programs and National Health Institutes.

The reporting system, with Ministry of Health entities as its main reporting sources, presents severe shortcomings regarding reliability in diagnosis, type of data collected, and data entry systems. However, the data collected by the system is used as a national reference base for the quantification and situational diagnosis concerning communicable diseases requiring mandatory reporting.

Presently, a computerized network is being implemented to improve the entry data system at the Regional Health Units and at the central level. This will, in turn, contribute to a better reporting system.

Case reporting, specifically in leishmaniasis cases, is based on clinical diagnosis and is usually performed by non-physician personnel. Cases are obtained in a passive way. The reporting system does not consider differences in the clinical presentation of the disease. It records the reporting place, which is not the probable site of infection in many cases. Furthermore, it does not distinguish new infection cases from old or chronic ones, thus monitoring the incidence of the disease in the population is not possible.

Current Epidemiological Status of Leishmaniasis in Peru

Over the past four decades, data recorded by the National System of Epidemiological Surveillance from the Peruvian Ministry of Health shows that leishmaniasis case reporting and prevalence has undergone a large increase. Table 1 and Figure 1 show that average prevalence ranged between 6.8, 8.8 and 8.7 per 100,000 inhabitants in the 1950s, 1960s, and 1970s respectively. However, by the 1980s, prevalence of leishmaniasis almost doubled, reaching an average rate of 14.5/100,000 inhabitants. This could be in part attributable to improvements in the reporting system; however, indications are that the problem has really increased.

TABLE 1: Leishmaniasis in Peru, 1953 - 1990, Number of Cases Reported and Prevalence Rate x 100,000 Inhabitants

YEAR	Nº OF CASES	PREVALENCE RATE
1951	222	2.80
1952	512	6.40
1953	525	6.38
1954	527	6.24
1955	553	6.38
1956	541	6.08
1957	671	7.34
1958	821	8.74
1959	1,061	10.99
$\bar{X}$		6.80
1960	1,017	10.24
1961	1,003	9.82
1962	732	6.96
1963	623	5.75
1964	907	8.14
1965	937	8.17
1966	1,239	10.50
1967	1,156	9.53
1968	1,231	9.87
1969	1,109	8.64
$\bar{X}$		8.8
1970	1,371	10.39
1971	1,011	7.45
1972	1,213	8.69
1973	1,069	7.45
1974	1,246	8.45
1975	1,393	9.19
1976	1,538	9.88
1977	1,464	9.16
1978	1,488	9.07
1979	1,282	7.61
$\bar{X}$		8.7
1980	1,551	8.97
1981	2,352	13.25
1982	1,882	10.33
1983	2,313	12.36
1984	2,400	12.50
1985	2,722	13.82
1986	2,766	13.69
1987	3,019	14.57
1988	4,570	21.50
1989	5,212	23.92
$\bar{X}$		14.5
1990*	2,796	12.52

* Partial Information

SOURCE: Peruvian Ministry of Health, Office of Statistics and Data, General Epidemiology Office

LEISHMANIASES IN PERU PREVALENCE RATE x 100,000 POPULATION 1,953 TO 1,990*

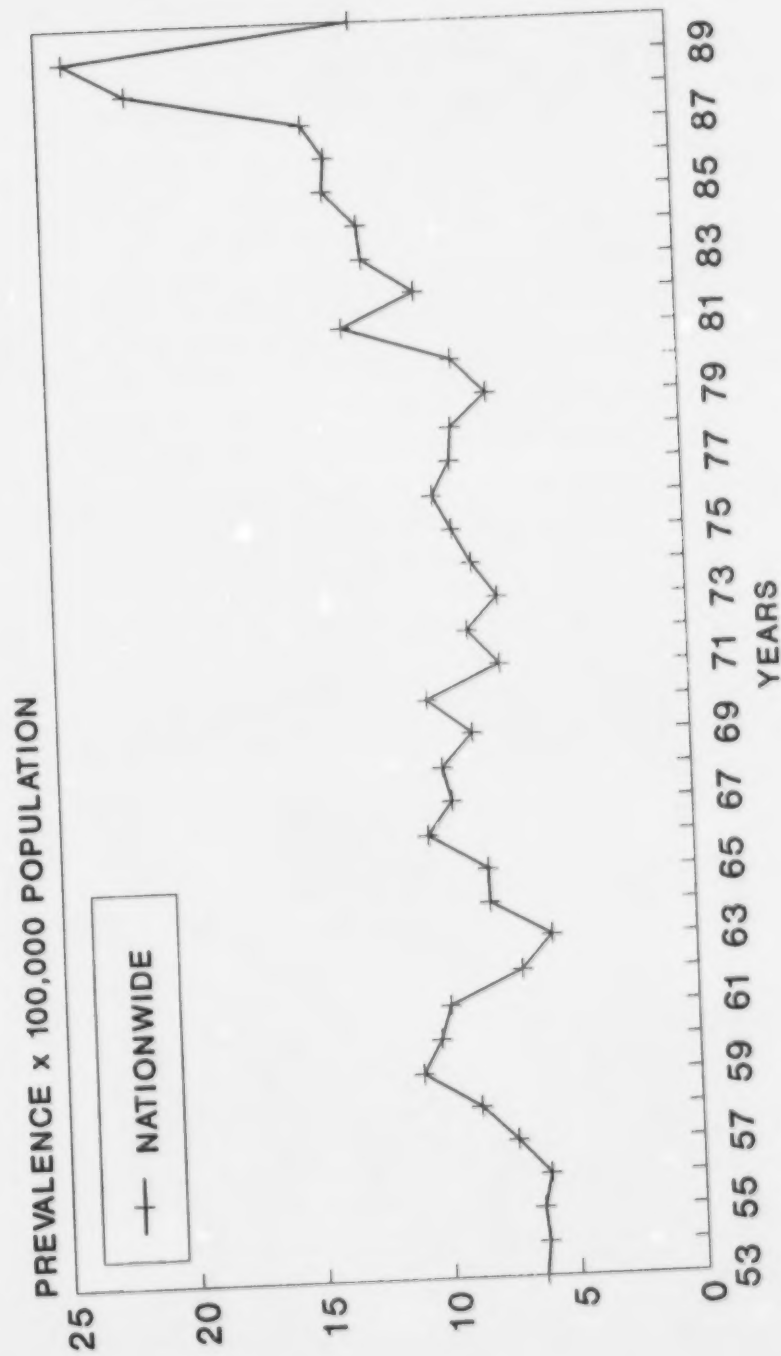


FIGURE 1

SOURCE: MINISTRY OF HEALTH OF PERU
*PARTIAL INFORMATION 1990

In 1989, leishmaniasis placed among the top ten primary causes of general morbidity in the country (Table 2). According to this year's statistical reports, 5,212 people suffering from leishmaniasis sought treatment at Ministry of Health centers. Although there is high underreporting of cases (estimated at greater than 40%) due to a variety of factors (including the system's own shortcomings, absence of active searching for cases and cultural patterns which view leishmaniasis as an inevitable event in the life of the Andean inhabitant), these figures provide evidence of the current magnitude of leishmaniasis in Peru. Leishmaniasis should be considered a serious public health threat and needs to be confronted in an integrated manner by means of a national control program.

TABLE 2: Ten Main Causes of General Morbidity in Peru - 1989

ORDER	REPORTING DISEASE	Nº OF CASES
1	Acute Respiratory Disease	670,833
2	Acute Diarrhoeal Disease	278,687
3	Intestinal Parasitosis	42,827
4	Malaria	36,329
5	Scabies	35,442
6	Tuberculosis	26,940
7	Typhoid fever	16,373
8	Chicken pox	13,567
9	Hepatitis	6,881
10	Leishmaniasis	5,212

SOURCE: Peruvian Ministry of Health, Office of Statistics and Data,
General Office of Epidemiology

Sixty-six percent of Peruvian provinces (120/183) have reported cases of leishmaniasis in the last 5 years. However, not all of these provinces correspond to transmission areas. The population in this area is estimated at 9.9 million for 1990.

Map N° 1 shows areas with a prevalence higher than 500 cases/100,000 inhabitants. Except for the Andean area of Huanuco (Province of Ambo), they are all part of the natural region of the lower jungle or Omagua, located between 80 to 400 meters above sea level. The weather is hot and humid, with plenty of rain. This area is rich in wild fauna, as well as lumber, rubber, chestnuts and gold mines on river shores of the departments of Madre de Dios, Ucayali and Puno. This area evidences open transmission and a high migration rate.

Map N° 2 shows the areas with a prevalence between 101-500 cases/100,000 inhabitants. These are low and high jungle or Rupa-Rupa areas, located between 400 to 1000 meters above sea level. Coca leaf crops are abundant in this region, characterizing a high rate of migration. This area also includes the Andean valleys of the

Yunga region at 500 to 2300 meters above sea level, where agriculture is the main occupation.

The areas shown on **Map N° 3** report a prevalence of 51 to 100 cases/100,000 inhabitants. This area includes low jungle, Yunga and Puna regions at 4,000 to 4,800 meters above sea level. The populations in this latter area live in extreme poverty, which forces people to migrate, mainly to the jungle areas with high transmission rates. In the course of performing extracting or collecting tasks in this area, health personnel thus expose themselves to the risk of acquiring leishmaniasis.

Map N° 4 shows the most extensive reporting areas, those with a leishmaniasis prevalence equal to or less than 50 cases/100,000 inhabitants. Many of the cases reported from these areas indicate the origin of the reporting but not the site of infection.

Table 3 shows case reporting of leishmaniasis between 1975 and 1990, according to various age groups.

TABLE 3: Leishmaniasis in Peru, 1953-1990, Cases Reported According to Age Groups

YEARS	AGE GROUPS (YEARS)						
	0-4	5-14	15-24	25-44	45-64	65 +	IGN
1975	70	173	403	530	191	24	2
1976	74	167	411	609	241	36	0
1977	71	239	396	528	198	30	2
1978	77	209	416	509	235	41	1
1979	43	196	391	469	150	31	2
1980	69	218	550	522	164	26	4
1981	130	356	795	801	243	41	4
1982	108	295	583	690	194	42	8
1983	157	435	648	748	260	60	4
1984	241	406	660	765	279	58	2
1985	259	466	915	908	312	61	2
1986	249	545	762	810	327	74	2
1987	298	695	846	996	409	78	0
1988	472	1,073	1,338	1,341	530	148	2
1989	488	1,170	1,350	1,447	621	109	9
1990*	304	542	783	756	333	76	2

* Partial information

SOURCE: Peruvian Ministry of Health, General Epidemiology Office,
General Office of Statistics and Data

MAP NO 2



LEISHMANIASES IN PERU
GEOGRAPHIC DISTRIBUTION
PREVALENCE RATE: 101 TO 500/100,000

MAP NO 1



LEISHMANIASES IN PERU
GEOGRAPHIC DISTRIBUTION
PREVALENCE RATE: > 500/100,000

MAP NO. 4



MAP NO. 3



Proportional distribution of the cases according to age groups (Table 4) shows that cases in groups 0-4 and 5-14 years have substantially increased.

In the 0-4 year-old age group, the average proportional distribution for the quinquenniums 1975-1979, 1980-1984 and 1985-1989 was 4.64%, 6.47% and 9.17% respectively; and for the 5-14 year-old group, it was 13.79%, 16.02% and 20.19% respectively. This may indicate that transmission at an early age is increasing (Figure 2).

Likewise, figures show that more than 50% of the cases are of individuals between 15 and 44 years old; that is young adults in the midst of social and economic activity. This evidences the probable association between the disease and the work-related tasks and activities which are performed. National statistics do not register data regarding sex, occupation, or social and economic status in leishmaniasis cases. These may be important factors associated with the risk of acquiring leishmaniasis, and they should be studied.

TABLE 4: Leishmaniasis in Peru, 1975-1990, Proportional Distribution According to Age Groups

YEARS	AGE GROUPS (YEARS OLD)							TOT
	0-4	5-14	15-24	25-44	45-64	65 +	IGN	
1975	5.03	12.42	28.93	38.05	13.71	1.72	0.14	100.0
1976	4.81	10.86	26.72	39.60	15.67	2.34	0.00	100.0
1977	4.85	16.33	27.05	36.07	13.52	2.05	0.14	100.0
1978	5.17	14.05	27.96	34.21	15.79	2.76	0.07	100.0
1979	3.35	15.29	30.50	36.58	11.70	2.42	0.16	100.0
1980	4.44	14.04	35.42	33.61	10.56	1.67	0.26	100.0
1981	5.49	15.02	33.54	33.80	10.25	1.73	0.17	100.0
1982	5.63	15.36	30.36	35.94	10.10	2.19	0.42	100.0
1983	6.79	18.81	28.03	32.35	11.25	2.60	0.17	100.0
1984	10.00	16.84	27.37	31.73	11.57	2.41	0.08	100.0
1985	8.86	15.94	31.30	31.06	10.67	2.09	0.07	100.0
1986	8.99	19.68	27.52	29.25	11.81	2.67	0.07	100.0
1987	8.97	20.92	25.47	29.98	12.31	2.35	0.00	100.0
1988	9.62	21.88	27.2825	27.35	10.81	3.02	0.04	100.0
1989	9.48	22.53	.99	27.86	11.96	2.10	0.17	100.0
1990*	10.87	19.38	28.00	27.04	11.91	2.72	0.07	100.0

* Partial information

SOURCE: Peruvian Ministry of Health, Office of Statistics and Data,
General Office of Epidemiology

Prevalence of leishmaniasis in the various age groups over the same time period is shown in Table 5 and Figures 3 and 4.

LEISHMANIASES IN PERU PROPORTIONAL DISTRIBUTION OF CASES BY AGE GROUPS AND QUINQUENIUMS

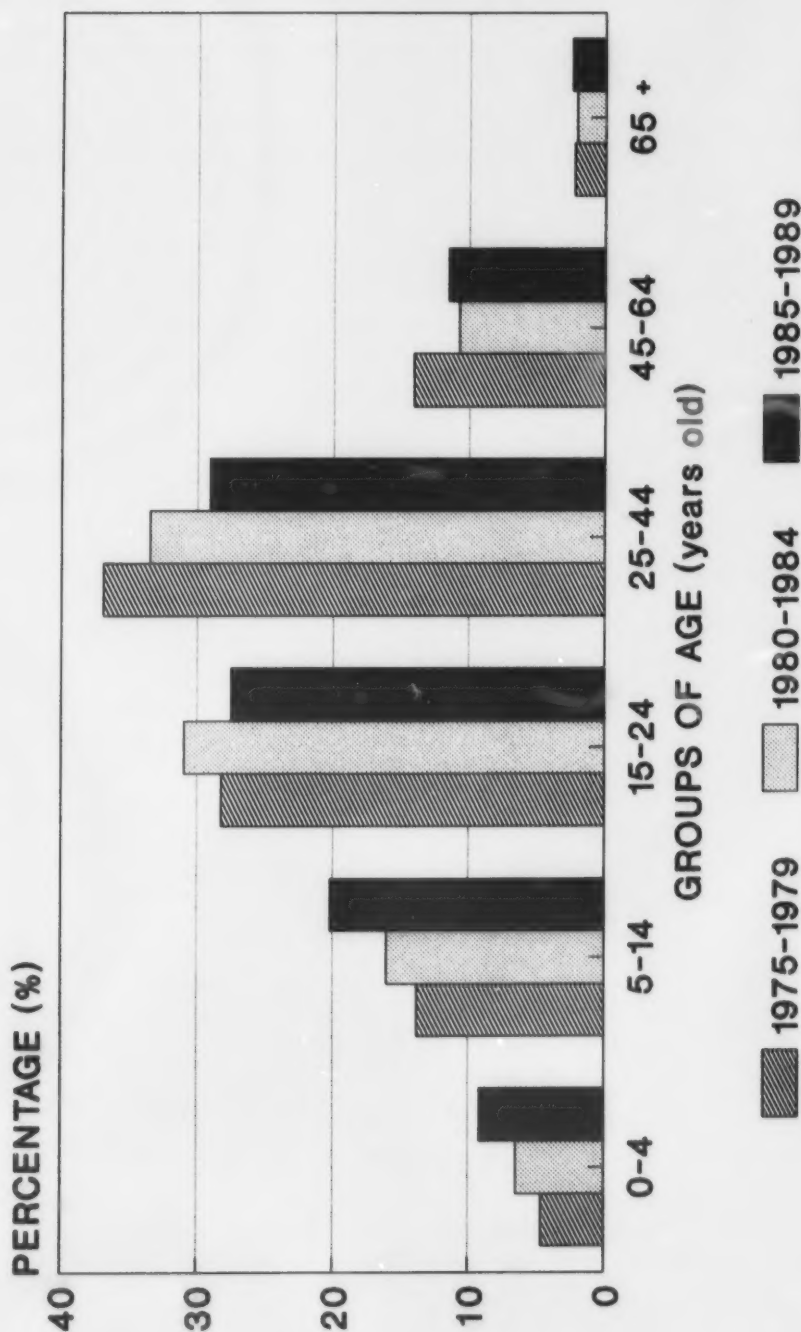


FIGURE 2

LEISHMANIASES IN PERU 1975-1990* PREVALENCE x 100,000 POPULATION BY AGE GROUPS

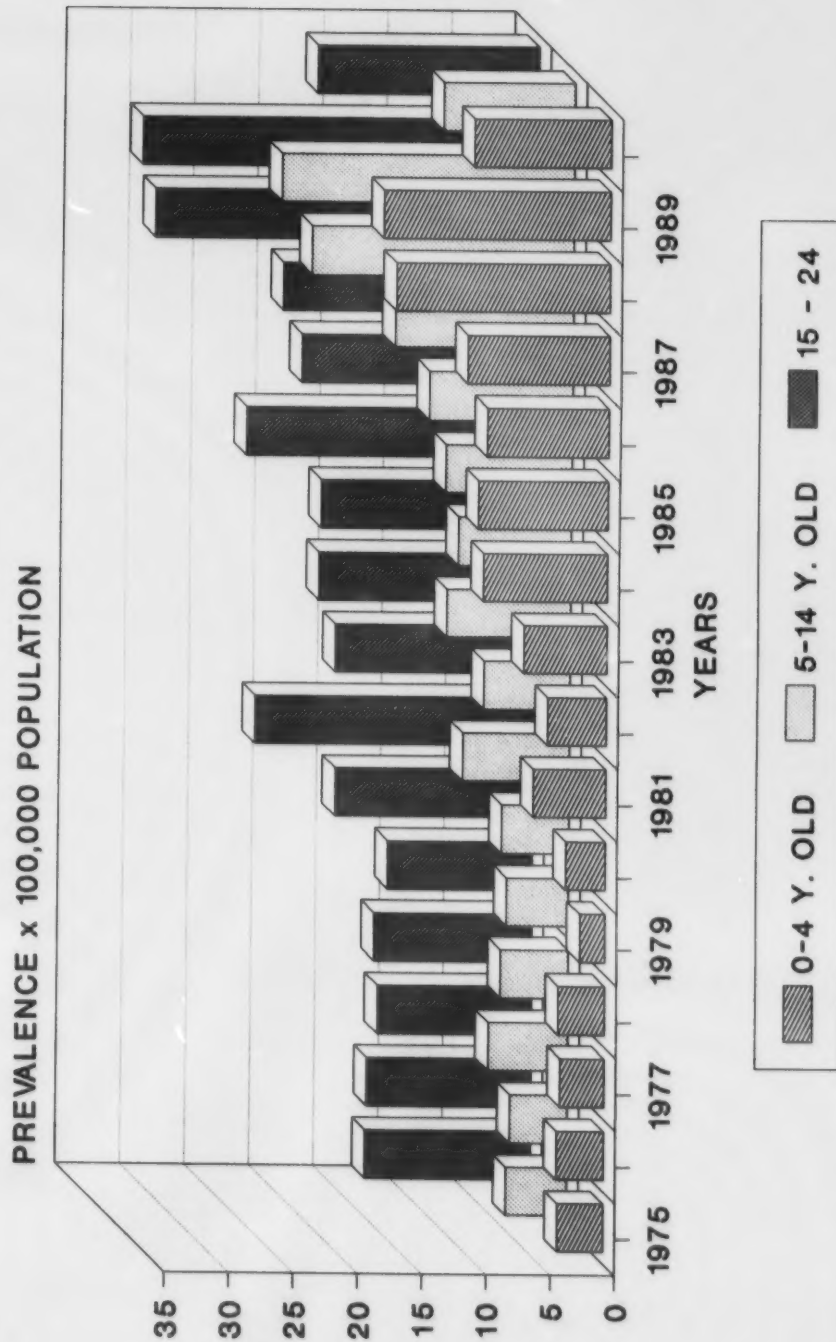
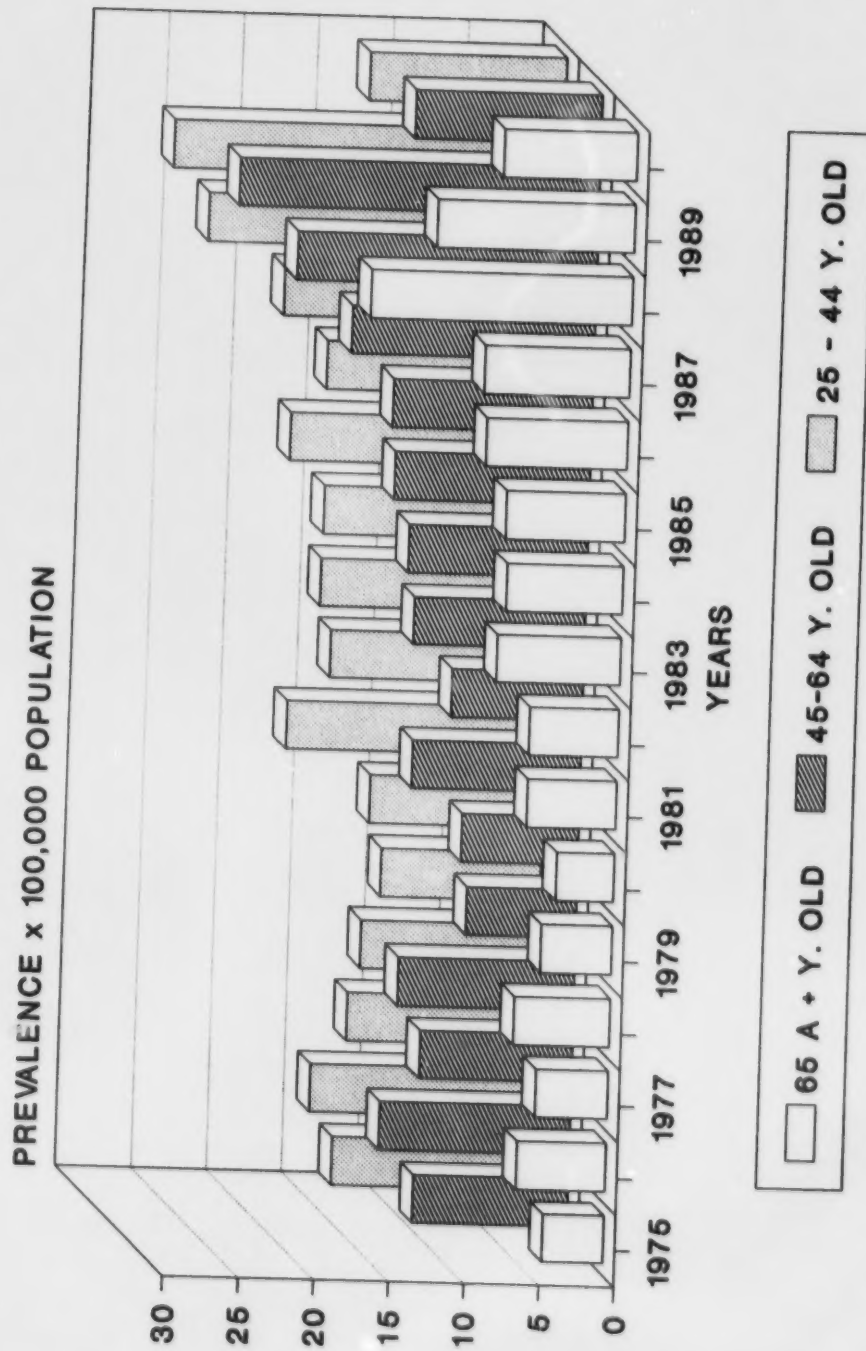


FIGURE 3

*PARTIAL INFORMATION - 1990

LEISHMANIASES IN PERU, 1975-1990* PREVALENCE RATE x 100,000 POPULATION BY GROUPS OF AGE



*PARTIAL INFORMATION 1990

FIGURE 4

In 1989, the prevalence of leishmaniasis was 30.83, 25.98, 23.95, 22.84, 17.66 and 41 cases per 100,000 inhabitants, among the age groups of 15-24, 25-44, 45-64, 5-14, 0-4 and 65 years old or more respectively. Those most affected also are the most economically active groups. The serious increase registered in the 5-14 year-old group in comparison with former years is an aspect of particular concern.

TABLE 5: Leishmaniasis in Peru, 1975-1990, Prevalence Rates x 100,000 Inhabitants According to Age Groups

YEARS	AGE GROUPS (YEARS OLD)					
	0-4	5-14	15-24	25-44	45-64	65 +
1975	3.55	4.73	12.90	13.34	10.33	4.06
1976	3.66	4.45	12.81	14.93	12.68	5.93
1977	3.42	6.20	12.02	12.60	10.15	4.81
1978	3.61	5.28	12.30	11.84	11.73	6.40
1979	1.96	4.83	11.27	10.62	7.30	4.72
1980	3.07	5.23	15.44	11.52	7.77	3.85
1981	5.63	8.32	21.74	17.22	11.22	5.92
1982	4.56	6.72	15.53	14.45	8.72	5.91
1983	6.46	9.65	16.82	15.26	11.39	8.22
1984	9.66	8.78	16.69	15.21	11.91	7.75
1985	10.11	9.82	22.55	17.59	12.98	7.94
1986	9.48	11.19	18.31	15.30	13.26	9.39
1987	11.06	13.91	19.81	18.34	16.17	9.65
1988	16.66	20.43	29.81	23.49	19.94	17.41
1989	17.66	22.84	30.83	25.98	23.95	13.15
1990*	10.63	10.22	17.28	13.12	12.41	8.86

* Partial information

SOURCE: Peruvian Ministry of Health, Office of Statistics and Data,
General Office of Epidemiology

Since the reporting system does not discriminate leishmaniasis according to its clinical presentation, it is very hard to evaluate the magnitude of harm using prevalence over time as a variable. However, 82% of the reports in 1989 corresponded to cutaneous leishmaniasis and 18% to mucosal/mucocutaneous leishmaniasis (Figure 5). The departments constituting this latter percentage were: Cusco (35%), Huánuco (34%), Madre de Dios (10%), Loreto (5%) and Ucayali (5%) (Figure 6).

LEISHMANIASES IN PERU - 1989
CASE NOTIFICATION BY CLINICAL FORMS

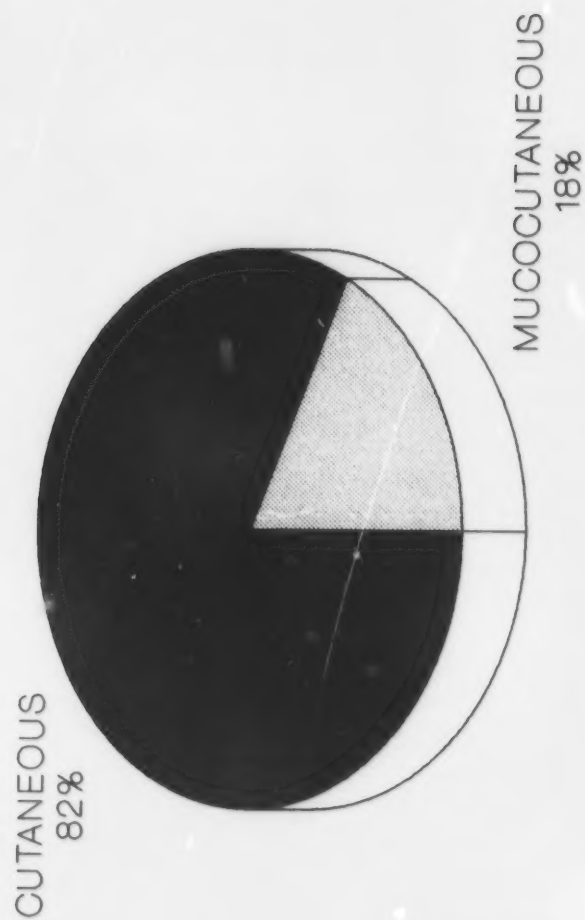


FIGURE 5

LEISHMANIASES IN PERU - 1,989 REPORTING AREAS OF L. MUCOCUTANEOUS

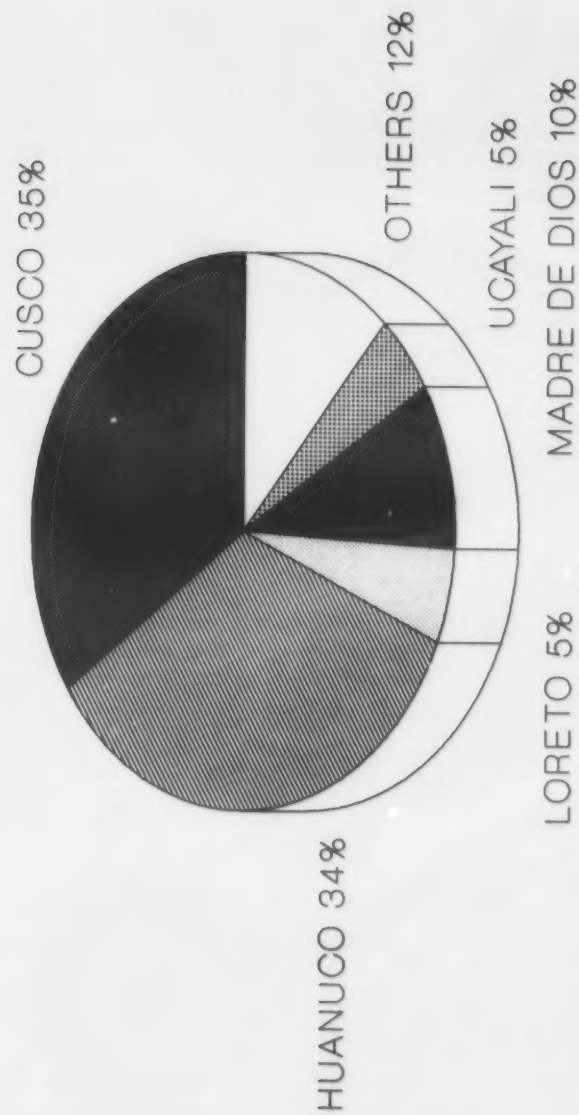


FIGURE 6

National Program for the Control of Leishmaniasis

The increased reporting of cases of leishmaniasis (especially among infantile and young adult groups), the wide geographic distribution of the disease, its negative social and economic impact on working capacity and productivity, combined with the suffering of patients who are mentally as well as physically incapacitated and the high cost of available treatment, are all indicators that the magnitude of leishmaniasis in Peru represents a serious public health threat.

To deal with this problem, it is essential to have a national program oriented towards the complete management of leishmaniasis with intra and intersectoral participation of all public and private groups, local and foreign non-governmental organizations, the church and the community.

The Peruvian Ministry of Health, by means of the National Program for the Control of Malaria and other Vector-Borne Diseases, has been carrying out several leishmaniasis-related activities since 1985. Initially these consisted mainly of the purchase of Glucantime and its distribution to the health facilities in order to provide free treatment to patients.

The National Program for the Control of Malaria and other Vector-Borne Diseases, during the period 1987-October 1991, has distributed 537,140 ampules of Glucantime, with an approximated expenditure of US\$ 2,000,000.

TABLE 6: Distribution of Glucantime in Peru, 1987 - 1991

YEAR	GLUCANTIME (AMPULE)
1,987	150,000
1,988	128,480
1,989	83,365
1,990	30,295
1,991*	145,000
TOTAL	537,140

* Data to October 1, 1991; Approximate cost: US\$ 2,000,000

Budget shortcomings, lack of trained human resources, the absence of standardized technical guidelines to support and to evaluate the approaches of a National/Regional Plan for the Control of Leishmaniasis, the need of adequate reporting system, the absence of multisectoral interest in the problem, were the main obstacles to be encountered.

In 1987, the Staff of the National Program for the Control of Malaria and other Vector-Borne Diseases called upon the various institutions that worked in isolation on

different topics of leishmaniasis, such as universities, research institutes, national and international non-government organizations and the church, to assist in the design of a National Plan. There was a successful and prompt response to this invitation and they agreed to work together with the health authorities to address a proposal for a National/Regional Plan for the Control of Leishmaniasis.

As a result of this joint effort, experiences and knowledge were shared and the first technical guidelines of plausible control measures for leishmaniasis were established by means of a standardized and decentralized treatment of cases, by levels of complexity of health facilities (community-sanitary post, health centers, hospital and specialized research institutes) and by clinical forms of the disease.

At that time, two models of primary health care strategies for the control of leishmaniasis were in use, one of them in Ambo, an endemic area of transmission and the other one in the high provinces of Cuzco, non-transmission areas.

Due to the high incidence and prevalence of leishmaniasis in Ambo, the regional authorities took the decision to confront the problem as a public health need. An amount of money was assigned in order to implement and develop a control plan. Technical support was requested from the National Program for the Control of Malaria and other Vector-Borne Diseases.

As a result, the Ambo health center became a decentralized pioneer treatment center, with the invaluable participation of a physician from the Tropical Medicine Institute of Cayetano Heredia University who went to work in the affected area for several months, despite the severe problem of terrorism existing in the zone. Professional and auxiliary health personnel were trained in the early diagnosis and timely treatment of leishmaniasis cases. The surveillance and notification system was enhanced through the passive and active detection of cases. Entomological studies were carried out and peri and intradomiciliary spraying of the houses with DDT was done.

In the high provinces of Cuzco, patient associations were organized. Most of them had become infected while working in gold mining and in extractive activities in the jungle of Madre de Dios. These associations started because of the patients' will and received active support from the church and non-government organizations. Non-government organizations in the area were mainly dedicated to agriculture and to the social development and promotion of human life. However, as a consequence of the poor response received from the health authorities to the solution of this problem, these organizations became active supporters of the leishmaniasis patient association.

This claim for help was attended by the regional authorities and the National Program for the Control of Malaria and other Vector-Borne Diseases, giving birth to the experience of decentralized community-based treatment for the control of leishmaniasis.

These experiences of primary health care strategies for the control of leishmaniasis were reviewed and analyzed in September 1990 at a workshop sponsored by the National Program for the Control of Malaria and other Vector-Borne Diseases which was held in Cusco. All the Peruvian leishmaniasis working groups from the various sectors participated in this meeting, government and non-government, including members of the associations of leishmaniasis patients. The aim was to build up a program for the control of leishmaniasis in the Inca Region and to establish the foundation for the design of a National Program for the control of the disease.

The following were identified as the main objectives for a National Plan for the Control of Leishmaniasis:

- i) To become aware of the real magnitude of the problem.
- ii) To decrease the prevalence of leishmaniasis.
- iii) To reduce the frequency of severe leishmaniasis cases (mucosal cases).
- iv) To provide the necessary logistic and technical capacity to carry out decentralized early diagnosis and timely treatment for leishmaniasis cases, starting at primary levels of community health care, with priority on the most affected areas and population groups.

To address these needs, the following strategies were suggested:

- Passive detection of cases in all of the country's health care facilities. These cases shall be treated according to standard methods and reported using proper formats.
- Active detection of cases by searching for patients in their own communities. These cases shall be treated and reported, as specified above, with the active participation of the community, health promoters, health personnel and other involved institutions.
- Establishing and implementing sentinel surveillance centers in selected risk areas, which will enable better information about the dynamics of the transmission and the associated risk factors to be obtained.
- Provide appropriate training to professional and auxiliary health personnel and community health agents in the epidemiological surveillance, early diagnosis and timely treatment of leishmaniasis. A continued educational program should be maintained.

- Implement and ensure financing and logistics, as well as the necessary equipment to allow for continuity and permanence of the control program in affected areas.
- Reinforce sanitary education among risk groups, providing them with the necessary information regarding the importance of early diagnosis and timely treatment of leishmaniasis.
- Monitor, supervise and evaluate the impact of the various components of the control program.
- Promote and support investigations, especially research that may lead to better knowledge of the epidemiological patterns in the transmission of leishmaniasis, as well as its real incidence and prevalence in various affected areas in the country.

Discussion Summary

Dr. Zarowsky emphasized the existence of different points of view, which have as a consequence different and often controversial approaches to facts, among people practising different disciplines. This comes to be particularly relevant when bio-medical and social scientists interact. The need to define and apply a multidisciplinary approach to the control of diseases requires an open minded attitude from both biomedical and social scientists involved in research as well as in executive aspects of control (e.g. at the MOH's). Excessive zeal or rigor by any of those working in either type of science is inadequate. It was also pointed out that a rather frequent attitude among researchers - which could be said to be common to health care providers also - is that they neglect the need to recognize the community's (and/or individual's) characteristics when planning and carrying out their activity or service.

Dr. Ivan Velez briefly described ecoepidemiology, and mentioned it was a multidisciplinary approach to the identification of weak links in the transmission chain. Among the basic steps were:

- *Identification of endemic foci.*
- *Mapping of cases and of important factors related to transmission, including their distribution in space (horizontal and vertical) and in time.*
- *Identification of critical values (e.g. critical vector density) that could be considered thresholds for decision making for control purposes.*
- *Awareness of the different conditions peculiar to every transmission focus.*
- *The implementation of a "permanent" program aiming to reduce incidence and prevalence of the disease, making it not simply a public health problem.*
- *The program should be focused and implemented with the participation of the community, whilst being integrated and global.*

People's habits and customs should be very much taken into account, since they relate the human community with the rest of the environment components. Control should not be only a responsibility of the government, but also of the community. A key factor for achieving community participation is to provide community members with knowledge on the disease and control possibilities.

Some concern was given to problems that ecoepidemiology could face with environment and wildlife integrity. It was reminded that concepts such as feasibility, compliance, sustainability, etc. should be always kept in mind by epidemiologists.

The presentation of Dr. Becerra depicted a primary health care based program that may be adapted to include leishmaniasis among the health problems it faces. Key elements in the approach described are the identification of the different co-participants; for instance, "the school for mothers", "the municipal government", and "the organized community", the

latter composed of family leaders, health promoters, volunteers, grassroots organizations, and others.

The actions should be horizontal and based on consensus and consultation (as opposed to vertical and imposed), and must involve the utilization of appropriate technology and the transfer of the methodology, both which should be understood by the community and felt as their own. In some way it means to "give people power".

The model was considered particularly applicable where leishmaniasis was not a priority health problem. In such cases, leishmaniasis should be considered part of a comprehensive PHC package.

Ms. Rodriguez presented the strategy implemented by the National Direction for Control of Malaria and Other Metaxenic (vector borne) diseases of the MOH of Peru. It has issued a call to a number of academic and service institutions working in some way in leishmaniasis, and formed a working committee that could act on a basis of consensus, multidisciplinary scientific rationale, and collaboration. This has permitted a close collaboration and transference of information, knowledge, and advice between the National Program and other institutions. A shortcoming of this has been that no national guidelines have been issued yet.

The implementation of a workshop on leishmaniasis control for Peru, held in Cusco in September 1991, with the participation of representatives of all the academic institutions engaged in research on leishmaniasis, NGOs, among others, is a representative feature of the multidisciplinary way problems can be approached.

The presentations made evident that many countries share problems such as under-reporting, lack of follow-up on patients, limited availability of material resources, and insufficient preparation of all level health workers to deal with leishmaniasis, among other diseases.

Observations on the Ecology of Visceral Leishmaniasis in Jacobina, State of Bahia, Brazil (1982 to 1986)

I.A. Sherlock¹ & J.C. Miranda¹

Introduction

This is a report on the project entitled "Ecology of visceral leishmaniasis in Jacobina, Bahia", which received financial support from the International Development Research Centre, Canada (IDRC) with the agreement of the "Fundação Oswaldo Cruz" and "Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil". The project was carried over the years 1982 to 1986, with some interruptions due to administrative and bureaucratic problems.

The specific aims of the project were:

- a) to compare the importance of sylvatic and domestic reservoir hosts in the transmission of visceral leishmaniasis in Jacobina, Bahia;
- b) to obtain information on the behaviour and vectorial capacity of vectors in Jacobina;
- c) to observe the importance of the human reservoir in the cycle of the transmission of visceral leishmaniasis in Jacobina.

I. The Study Area

Jacobina is one of the oldest known and most important foci of visceral leishmaniasis in the American continent. It is a city located in the county of Jacobina in the interior of the State of Bahia, Brazil at 11°11'08" S latitude and 40°31' W Gr longitude, 465 m above sea level, in the zone of which is climatically prone to drought. The physiography of the area is composed of mountains and elevated plains. The Itapicuru river runs through the mountains and receives several tributaries, among them the "rio do Ouro", that passes through the city of Jacobina. The mean annual rainfall is 550 mm and the mean annual temperature is 24°C, ranging from the minimum 24°C to the maximum 31°C (12). About three decades ago the area of Jacobina was forested. Presently, primary vegetation of tall trees is rare due to indiscriminate deforestation. In those forests of the past lived several species of sylvatic animals, including mammals which are now restricted to a few specimens and belong to a small number of species.

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The soil is rich in minerals such as gold, saltpetre, silica, copper, amianthus, iron, manganese and uranium (12). Nowadays, these are exploited by multinational companies benefitting from the cheap manual labor of the inhabitants of northeast, who are attracted to the area looking for work. This is the principal reason, together with the drastic conditions of the climate, for the intense migration that exists in Jacobina. This fact has a deep influence on the dispersion and distribution of the visceral leishmaniasis (9,27).

Granitic and calcareous rocks are very abundant, often forming caves that are common in the area of the geographical distribution of the Lutzomyia longipalpis, the principal vector of the American visceral leishmaniasis (9,27).

In 1984, the estimated population of the municipality of Jacobina was 50,000 inhabitants and 83% of them lived in the rural area. Ethnically the population was composed principally of mestizos. Badaro (5) provides a bibliographic review of the clinical and epidemiological aspects of visceral leishmaniasis in Jacobina.

According to the first case registration by Sherlock (27) in 1959, the incidence of visceral leishmaniasis does not show any difference in relation to the sex or race of the patients. However, there were pronounced differences depending on age. Children from 0 to 5 years old were attacked more than adults. This fact has been corroborated with findings in other endemic foci in the northwest of Brazil.

The per capita income of inhabitants of the peripheral sites of Jacobina is very low. As the city grows, the poor population is being pushed far from the periphery once again.

The most common type of dwelling is one that is built with stones or bricks. These buildings can be considered of good standard when compared with those in other endemic areas, where they are predominately made of mud bricks or sticks and mud. Probably, for this reason, Jacobina is practically free of the proliferation of the insect vectors of Chagas' disease. Consequently, there are not any autochthonous human cases of Chagas' disease in Jacobina. It is, however, necessary to emphasize that type of housing does not have any importance in the epidemiology of visceral leishmaniasis (27, 29, 35).

The city of Jacobina was arbitrarily divided into 7 sub-areas, with different incidence rates of human cases, based on the work of Teixeira (40). The sub-area "Grotinha" was selected for our observations because human cases of visceral leishmaniasis have been occurring there in the last three years. Our 1982 census in "Grotinha" showed 323 dwellings where 1,128 inhabitants lived.

It is important to emphasize that the population of Jacobina is constantly increasing with individuals that emigrate from other areas, including those endemic areas of visceral leishmaniasis. Some individuals also emigrate from Jacobina to other states, principally to São Paulo. Thus, from time to time, the specialized bibliography mentions human cases of visceral leishmaniasis in São Paulo.

The name "Grotinha" means little valley. There is a disorderly row of houses along a tortuous way between two mountains. At the end of this valley, there is still some vegetation, composed of shrubs and small trees of secondary formation. The consequences of years of uncontrolled deforestation, which remains very intensive even now, is that, continuous erosion exists in the hills around the city. Siliceous and calcareous stones roll from the hills and drop near houses. These stones are useful in building houses. They also represent an most important economic source for many of the inhabitants, because they break the stones and sell them to other areas. Both adults and children do this work until late, outside houses, increasing their contact with the peridomestic vector.

During the four years of our observations on visceral leishmaniasis, there was active transmission of the disease, although in the first two years, few human cases were detected. In the two subsequent years an epidemic was verified when more than 50 cases were registered. According to Badaro (4) the annual global incidence (1980 to 1984) was 4.3/1000 children, but in Grotinha the incidence was 13.7/1000 children.

Analysis of risk factors for the development of the disease, according to Badaro (4), revealed that the age 0 to 5 years as the principal factor, followed by bad nutritional status. Thus, 75% of the malnourished children, when infected developed severe symptoms. The serological rate for positive antibodies, predominates in children above 5 years of age. Another important observation of Badaro was the confirmation of seropositive children with visceromegalia but without the manifest symptomatology of the disease. There exists a greater number of asymptomatic seropositive children than those with typical clinical disease.

The general epidemiological conditions of visceral leishmaniasis in Grotinha are shown in the Table I. As one can see from the table the area is characterized by a large number of infected dogs, a high rate of people with visceral leishmaniasis, and a high rate of sandfly infested houses.

TABLE I
NUMBER OF HOUSES WITH PATIENTS EXAMINED, SICK DOGS AND
SANDFLIES IN GROTIHA, A SUBURB OF THE CITY OF JACOBINA, BAHIA
(1982 to 1984), ENDEMIC AREA FOR VISCERAL LEISHMANIASIS

Houses	Number	Percentage
Total Inhabitants	1137	100
Number of houses	323	100
Houses surveyed for sandflies	299	92
Houses infested with sandflies	63	21
Houses with dogs examined	62	19
Houses with infected dogs	7	11
Houses with Kalazar patients	26	8

II. Etiologic Agent

Methods - The specific leishmanias identification was made by zymodeme analysis based on its characteristic reactivity with species specific monoclonal antibodies using either an indirect radioimmune binding assay or immunofluorescence and immunoperoxidase technique (30). The behaviour of the leishmanias was observed in cultures, in the digestive tract of *L. longipalpis* and in the challenge of hamsters by intraperitoneal and intracutaneous inoculation of leishmanias. Histopathologic, immunological, biochemical and molecular biology techniques are described by Sadigursky (23) and Grimaldi (30) who were responsible for the realization of such techniques for this work.

For the diagnosis of the infected hosts and the isolation of the parasites, besides IFT we have employed other techniques such as culture of leishmanias in several media (NNN, LIT, RPMI, BHI), Giemsa stained smears of organs, inoculation of macerates of organs into hamsters and posterior confirmation of the hamster infection six months later end histopathologic study of organs. Xenodiagnosis were made with laboratory bred *L. longipalpis*. Human cases were diagnosed by Dr. Badaro's clinical staff in Jacobina, by means of the clinical symptomatology and isolation of leishmanias from bone marrows and spleens.

Results - The following species of trypanosomatids were found in Jacobina: Leishmania chagasi (8) infecting man, dog and opossum; Leishmania amazonensis Lainson & Shaw, 1972 infecting man and opossum; Leishmania guyanensis Vianna, 1911 (sub sp.) infecting opossum; bodies like leishmania amastigotes, that could not be specifically identified, were seen in the aguti Dasyprocta aguti and in the sylvatic rodents Cercomys cunicularius and Oryzomys eliurus, and in a domestic cat. Trypanosomatids other than leishmania were also found: Trypanosoma cruzi in the opossum Didelphis albiventris, and an epimastigote not specifically identified and not isolated, that we think to be Blastocrithidia, in three smears from the ear of a dog from Grotinha.

Leishmania chagasi was the causative organism of visceral leishmaniasis in man. But we have to emphasize that recently Barral et al. (6) have also been isolating only Leishmania amazonensis from the bone marrow of patients with the typical symptomatology of visceral leishmaniasis in Jacobina (5, 6). So, besides L. chagasi, this other species is also part of the ecology of american visceral leishmaniasis, at least in Jacobina. We tend to admit that the viscerotropic Leishmania amazonensis is a rather new species of leishmania pertaining to Jacobina.

The species of leishmania were maintained in hamsters and in several culture media, as mentioned before. L. chagasi was reisolated 89 times from the experimentally infected hamsters. The best culture medias were LIT and NNN. L. guyanensis (like) was reisolated 13 times and its best culture medium was BHI. L. amazonensis was reisolated 71 times and its best culture medium was NNN.

Direct isolation of L. chagasi from sylvatic hosts was not feasible. Prior inoculation of hamsters with macerates of spleens of the suspected animals is necessary to confirm and isolate the infection of the sylvatic host six months later. It seems that there is a specific dominance of the leishmania species that eliminate the weaker species from the culture media as follows: L. amazonensis domines, L. chagasi domines, L. guyanensis.

It was also possible to isolate L. chagasi through the xenodiagnosis with L. longipalpis from man, dog, opossum and hamster (Table V-VIII).

The behaviour of some species of leishmanias in the tract of L. longipalpis will be dealt with in detail in the section of this paper on infection of the vector.

III. Vector

Methods - Sandflies were caught in the morning, afternoon and at night inside houses, in chicken pens, pig pens, corrals, natural caves, tree trunks, etc., by means of direct localization with hand lights and suction with Castro's collector apparatus, and with a Falcao electric trap (34).

During 24 consecutive months the light traps were set at 6 p.m. and collected at 6 a.m. the following day, in a cave located near houses and where cocks were maintained as live bait. Inside the houses from 7 to 8 p.m., sandflies were collected off human baits. The collections were made for two days of every month during the years 1982 to 1984. Some of the collected sandflies were dissected and examined for flagellates and some of the engorged females were maintained alive in the laboratory for oviposition and maintaining of a laboratory colony of L. longipalpis. All the 49,048 sandflies collected were specifically identified.

Samples of several materials, suspected as possible breeding places of sandflies, were observed according to techniques described before (26). The methods for maintaining sandflies in the laboratory, for xenodiagnosis, feeding and infecting sandflies through membranes, were based on Sherlock (37) and Ward, Lainson & Shaw (43).

Results - Of the total 49,048 collected sandflies, 45,419 were L. longipalpis (Lutz & Neiva, 1912) (92%). Other collected species were Lutzomyia lenti Mangabeira, 1942, Lutzomyia evandroi (Costa Lima & Antunes, 1936), Lutzomyia trinidadensis (Newstead, 1922) and Lutzomyia oswaldoi (Mangabeira, 1942) (Table II).

TABLE II
SANDFLIES COLLECTED AROUND HOUSES AND CAVES IN JACOBINA,
BAHIA (1982 - 1986)

SPECIES	MALE	FEMALE	TOTAL
<u>L. longipalpis</u>	29,365	16,054	45,419(92%)
<u>L. lenti</u>	926	439	1,365
<u>L. evandroi</u>	1,101	652	1,753
<u>L. trinidadensis</u>	16	16	32
<u>L. oswaldoi</u>	2	5	7
<u>Lutzomyia</u> sp	254	218	472
TOTAL	31,664	17,384	49,048

In spite of the difficulties of obtaining successful transmission of L. chagasi by the bite of L. longipalpis this species is considered the principal vector of the parasite in the american continent (1,9,27). The basis for this is: coincidence of the geographical distribution of human and canine visceral leishmaniasis with one of these species; its natural and experimental infection with L. chagasi; its pronounced antropophilic habits, feeding frequently on man.

The formal proof of the transmission of L. chagasi by L. longipalpis, in spite of our persistent attempts was not acquired (35). In contrast, it was possible to transmit L. amazonensis by means of L. longipalpis bite (Table III and IV).

TABLE III
ATTEMPTS TO TRANSMIT LEISHMANIAS TO HAMSTERS THROUGH BITES
AND MACERATES OF L. LONGIPALPIS

TYPE OF INOCULATION	INFECTING LEISHMANIAS	HAMSTERS		No. of Positives
		No. Used	No. Examined	
Bites of Sandflies	<u>L. chagasi</u>	22	19	0
	<u>L. amazonensis</u>	9	9	6
Macerates of in- fected sandflies	<u>L. chagasi</u>	19	16	0
	<u>L. amazonensis</u>	2	2	1

TABLE IV
ATTEMPTS OF TRANSMISSION OF LEISHMANIA CHAGASI (FROM JACOBINA) TO HAMSTERS, BY THE BITE OF LUTZOMYIA LONGIPALPIS, OR INOCULATION OF MACERATES OF THESE INFECTED SANDFLIES, WITH NEGATIVE RESULTS

TYPES OF INOCULATION HAMSTERS	No OF EXAMINE\NEGATIVES
Bites of the suspected sandflies collected in Jacobina.	69
Macerates of naturally infected sandflies from Jacobina.	6
Bites of experimentally sandflies in amastigotes infected organs through membranes.	5
Macerates experimentally infected sandflies in infected organs mascerated, through membranes.	6
Bites on infected sadflies in dogs from Jacobina.	9
Macerates of infected sandflies in hamsters.	12
TOTAL	107

In Jacobina we found only the type of L. longipalpis with two spots (on the 3th and 4th tergites), but in a locality near Jacobina, just one spot L. longipalpis (on 3th segment) was the only species found. In this other locality, visceral leishmaniasis occurs sporadically. Ward et al. (44) suggested that L. longipalpis with two spots is probably the vector of visceral leishmaniasis in endemic areas, while L. longipalpis with just one spot is responsible for sporadic transmission. The two types of L. longipalpis have different pheromone composition in their spots, according to those authors (43). Once more, the population of L. longipalpis with two spots in Jacobina, is composed of two distinct types of individuals that can be distinguished by the naked eye: one big, dark and hairy and the other small, clear and hairless. The differences are possibly due to the ages of the individuals. L. longipalpis was found naturally infected with promastigotes, epimastigotes and tripomastigotes of non-specifically identified trypanosomatids in

Jacobina. It was also found naturally infected with ascocistes and microfilaria still not identified specifically.

There is a seasonal fluctuation in the density of L. longipalpis. This vector is more abundant during the colder and drier months and also in the hotter and rainier periods. The seasonal fluctuation correlates with the forthcoming of new human patients and with the increasing peridomestic frequency of the opossum Didelphis albiventris. The hourly activity of L. longipalpis begins at dusk and reaches its highest peak from 9 to 11 p.m. After 11 p.m. the number of sandflies decreases until the sandflies totally disappear at 6 a.m.

Two other species of sandflies are very much antropophilic, usually biting man, but preferring to feed on dogs; these species are L. lenti and L. evandroi. The first species was found naturally infected with promastigotes. Since they are usually found together with L. longipalpis in several focus of visceral leishmaniasis, there is some suspicion of their involvement in the transmission chain of american kalazar (25).

The natural breeding places of sandflies were not found in Jacobina. In the laboratory L. longipalpis is easily bred (37). During the present project some observations were done on its biology in the laboratory. We emphasized the development of a second batch of eggs from which adults were obtained. Engorged females can survive for more than 14 days. Species of Acari still not specifically identified and two species of fungi: Paecilomyces heliothis (Charles) and Cunninghamella elegans Lender (1907), destroy the laboratory colonies of L. longipalpis.

Lutzomyia longipalpis was easily infected with the following species of leishmanias: L. chagasi (human strain from Jacobina, Bahia and Imperatriz Maranhao; dog strain from Jacobina and Conde, Bahia; opossum strain from Jacobina, Bahia); L. amazonensis (opossum strain from Jacobina). It was not infected with human strain of L. braziliensis (Table X). L. longipalpis was also infected with L. donovani from India. Several species of animals served as blood source for the infection of L. longipalpis with L. chagasi such as man, dog, opossum and hamster. Experimentally infected mice infected well with L. longipalpis and with L. amazonensis. The behaviour of the promastigotes in the gut of L. longipalpis was that of characteristic suprapylarian position with L. chagasi and L. amazonensis, but this last species sometimes showed peripylarian position.

It was possible also to infect L. longipalpis with T. cruzi feeding on infected guinea pig. However, this trypanosome did not develop well in the gut of the sandfly (32).

Looking for the possibility of other existing vectors, we examined 136 specimens of Rhipicephallus sanguineus, 26 specimens of Ctenocephalides sp and 36 specimens of Stomoxys calcitrans collected feeding on five infected dogs in Jacobina. They were not found to be infected with leishmania.

TABLE V
XENODIAGNOSIS WITH L. LONGIPALPIS IN EXPERIMENTALLY INFECTED
HAMESTERS WITH DIFFERENT STRAINS OF L. CHAGASI

TYPES OF LEISHMANIA	No. XENOS REALIZED	+ve XENOS	No. SANDFLIES EXAMINED	+ve SANDFLIES
<u>L. donovani</u> (India)	05	01	82	10
<u>L. chagasi</u> (Dog) (Jac.\BA)	05	01	85	08
<u>L. chagasi</u> (Man) (Imp.\MA)	03	01	92	01
<u>L. chagasi</u> (Man) Risea (Jac.\BA)	01	0	20	0
<u>L. brasiliensis</u> (man\Bahia)	01	0	28	0
TOTAL	15	03	307	29

IV. Reservoirs

Domestic reservoirs

Methods - In Grotinha, only dogs and cats were examined because other domestic mammals like pigs, donkeys, horses, cows and goats were very rare. It was a pity because equines have been found to be infected with leishmanias in some foci of cutaneous leishmaniasis in Brazil (14).

In our 1982 survey we found 245 dwellings and 83 houses where there were domestic animals. Among these, 78 were dogs and 62 cats. The rest were represented by a few goats, 3 donkeys, 9 pigs and some chickens. Only dogs and cats were submitted to biopsies of ears for smears and the obtainment of drops of blood on filter papers for immunofluorescent testing (IFT) with *L. chagasi* antigen. The dogs were gagged, the ears cleaned and the point cut, in accordance with already described techniques (1,9,27). We proceeded in the same manner with the cats but it was necessary to contain them in canvas sacks.

All positive animals were brought to the laboratory in Salvador for a more detailed examination. This included the realization of xenodiagnosis with triatomines bugs and sandflies and submission to necropsy for histopathologic examination and culture of leishmanias from several organs, and inoculation into hamsters.

Results - Cats - Fifty three cats were examined with smears and IFT. In the skin of one cat, we found just one typical amastigote. This cat could not be re-examined and for this reason the specific identification of the amastigote was not made. All IFT were negative for leishmania.

As in other foci of Brazil it is possible that cats do not have any importance as a reservoir of *L. chagasi*. Cats almost never attract *L. longipalpis* and until now only one cat has been found naturally infected with a non specified leishmania. In an important focus of visceral leishmaniasis in the northeast of Brazil, Deane (9) examined 142 cats and all were negative for leishmania (Table VI).

TABLE VI
CATS EXAMINED FOR LEISHMANIASIS IN JACOBINA, BAHIA, THROUGH
SEROLOGICAL TEST AND STAINED EAR SMEAR EXAMINATION

TECHNIQUES	N0. EXAMINED	POSITIVES	%POSITIVES
Serology (IFT)	53	0	0
Ears smears	53	1*	1.9

* Only one typical amastigote was found in an ear smear from a healthy cat; it was not possible to re-isolate and definitely identify the flagellate.

Dogs - Two sero-parasitologic surveys were made with dogs in Grotinha. In 1982, 23.9% of the dogs were seropositive while at the same time a small number of human cases were occurring. In 1984 the seropositivity rate was 47.5% among dogs, and at the same time a human epidemic occurred (Table VII).

TABLE VII
DOGS POSITIVE FOR LEISHMANIASIS IN JACOBINA, BAHIA IN THE YEARS
1982 AND 1984, THROUGH SEROLOGICAL TESTS AND STAINED SMEARS.

D O G S E X A M I N E D *						
YEAR	S E R O L O G Y C A L T E S T			S M E A R S		
	(I F T)					
	EXAMINED	POSITIVE	%	EXAMINED	POSITIVE	%
1982	46	11	23.9	46	01	2.0
1984	40	19	47.5	42	05	12

* Those positive in smears were killed soon after the results.

Confirming anterior observations in Jacobina (27,28,29) and in other foci of Brazil (1-9), dogs also represent a better source for the infection of *L. longipalpis* than man does. In our experiments 78% of dogs infected 29% of the sandflies (Table VIII). Man infected only 15% of the sandflies in 33% of the attempts.

In edemic areas, because of their higher prevalence of infection and their infectivity for the vector, dogs are the most important domestic reservoir during epidemics, in spite of their being victims of the pathogenic action of the *L. chagasi* (1,9,28).

Man - The results obtained regarding the capacity of man to attract and infect the vector *L. longipalpis* were similar to those obtained by Deane (9) with patients of other foci. In our results 15% of the sandflies were infected by the six patients with visceral leishmaniasis who were submitted to xenodiagnosis with *L. longipalpis* in 2 of 6 attempts (33%) (Table VIII).

TABLE VIII
COMPARATIVE RESULTS OF THE CAPACITY OF LUTZOMYIA LONGIPALPIS
TO INFECT MAN, DOG AND OPOSSUM INFECTED WITH LEISHMANIA
CHAGASI FROM JACOBINA-BAHIA

Host Type	XENOS				SANDFLIES		
	Number	Realized	Positive	%	Examined	Infected	%
Man	2	6	2	33	201	32	15
Dog	8	20	13	78	368	107	29
Opossum	1	8	2	25	193	27	14

In the endemic area, the role of man as a reservoir is small, since man's capacity to infect the vector is poor. During epidemics, because of the increase in new cases and the higher density of L. longipalpis, the importance of the role of man as a reservoir, also increases due the existence of more opportunities for the infection of the vector. This fact was suggested by the greater occurrence of new cases after the peak of the density of L. longipalpis during its seasonal fluctuation.

Domestic rodents - The domestic rodents: Rattus rattus, Rattus norvegicus and Mus musculus were not studied in significant numbers in Jacobina, in spite of the former findings of the L. infantum infection in Rattus rattus from the Mediterranean (10-22). Also, years ago we speculated about their possible involvement in the chain of transmission of visceral leishmaniasis in Jacobina (29). Only 20 specimens of Rattus rattus were examined and none were found to be infected with leishmanias. The same happened with 42 specimens of Mus musculus.

Sylvatic reservoirs

Methods - Over four years, for a week every month, observations were made in Jacobina on sylvatic reservoirs. Forty wire traps for small animals and three for big ones, were set in strategic places to catch mammals, in the remaining forests of Grotinha. They were set in the morning and evening and collected the following day and the baits were changed. Fruit, cheese, corn, peanuts, etc, were used as bait. Pineapple was the best bait to catch opossums and pumpkin seeds were the best to catch wild rodents.

Besides the collected specimens of mammals, we acquired from local hunters specimens of foxes, agutis and armadillos caught around the city of Jacobina, that were studied here.

The species captured in Jacobina are very common in the State of Bahia. Thus, specific identification was made by the senior author, based on comparison of the taxidermized laboratory samples. These were previously identified by experts of the National Museum of Brazil. The specific identification of foxes as Lycalopex vetulus was made because they looked identical to those studied by Deane in the State of Ceará. In 1954 the senior author had the opportunity to work with Deane during the investigation of the visceral leishmaniasis in that focus (9).

The species Cerdocyon thous (= Dusycion thous?) studied by Lainson (13) in the State of Pará, Brazil, look bigger and darker than the specimens we have studied here.

All animals were submitted to xenodiagnosis with triatomines bugs and L. longipalpis and were examined by means of: fragment cultures of their organs in several media (NNN, LIT, RPMI, BHI); inoculation into hamsters of macerates of their spleens; stained smears of their spleen, liver and skin; hystopathologic examination of fragments of several organs and sometimes IFT of their sera.

Results - The sylvatic fauna - In Grotinha 3,353 traps were set during the years 1982 to 1984 in which only 166 (5%) caught 178 mammals specimens. The results do not mean, that the traps were inefficient but rather they showed that there was a very poor fauna with few species and specimens exists in the periphery of Jacobina. Only 11 mammal species were obtained, included two species of domestic rodents (Table IX).

TABLE IX
MAMMALS CAPTURED AROUND THE CITY OF JACOBINA, BAHIA (1982-1986),
FOR RESEARCH ON LEISHMANIAS

SPECIES OF MAMMALS	Nº CAPTURED	% TOTAL
<u>Dasyprot. aguti</u>	8	3.8
<u>Didelphis albiventris</u>	94	44.1
<u>Cabassous unicinctus</u>	6	2.8
<u>Cavia porcellus</u>	7	3.2
<u>Lycalopex vetulus</u>	14	6.6
<u>Cercomys cunicularius</u>		
<u>inermis</u>	11	5.2
<u>Mus musculus</u>	42	19.7
<u>Rattus rattus alexandrinus</u>	20	9.4
<u>Chiroptera</u>	1	0.5
<u>Oryzomys subflavus</u>	1	0.5
<u>Oryzomys eliurus</u>	9	4.2

The predominant species of mammal was the marsupial opossum Didephis albiventris Lund, 1841, that reached 44% of the total. Following this were rodents with a frequency of 39%. However, sylvatic rodents were less frequent than domestic ones. Only 14 specimens of foxes were obtained with much difficulty even though attractive financial offers were made to buy foxes.

Infected animals - A total of 213 specimens were studied. Among them 193 underwent all types of examination mentioned above. Besides the specimens infected with leishmanias that will be referred to later, bodies like leishmania promastigotes were seen in the smears of liver and spleens of four specimens of Dasyprocta aguti and in smears of spleen of a Cercomys cunicularius and a Oryzomys eliurus. Unfortunately they could not be specifically identified.

Among 94 specimens of D. albiventris that were studied we obtained results from 84, of which four were infected with three leishmania species and three with Trypanosoma cruzi (Table X). The species of leishmanias and the number of oposums were: two with Leishmania chagasi, one with Leishmania amazonensis and one with Leishmania guyanensis, sub-species.

TABLE X
WILD AND COMMENSAL ANIMALS OBTAINED FROM 1982 TO 1986 IN
JACOBINA, BAHIA FOUND NATURALLY INFECTED WITH
TRYPANOSOMATIDS

MAMMAL SPECIES	Exami- ned	I N F E C T E D			W I T H	
		L.cha- gasi	L.amazo- nensis	L.brazi- liensis sp	Bodie amasti- gotes	T.cruzi
<u>D. albiventris</u>	84	2	1	1	-	3
<u>Das. aguti</u>	7	-	-	-	4	-
<u>C. porcellus</u>	6	-	-	-	-	-
<u>C. cunicularius</u>	12	-	-	-	1	-
<u>O. eliurus</u>	8	-	-	-	1	-
<u>O. subflavus</u>	1	-	-	-	-	-
<u>R. rattus</u>	19	-	-	-	-	-
<u>M. musculus</u>	35	-	-	-	-	-
<u>Chiroptera</u>	1	-	-	-	-	-
<u>C. unicinctus</u>	6	-	-	-	-	-
<u>L. vetulus</u>	14	-	-	-	-	-
TOTAL	193	2	1	1	6	3

Human and canine infection with T. cruzi, that are not autochthonous in Jacobina, are rare, probably because triatomine bugs do not exist in the city.

Although D. albiventris is found infected with L. amazonensis and L. braziliensis, cutaneous leishmaniasis is not usually found in the city. However, we have to emphasize that L. amazonensis has been found in several patients with visceral leishmaniasis by Barral et al. (6), as mentioned before. Lutzomyia flaviscutellata, the principal vector of L. amazonensis was not registered in Jacobina. But other species belonging to its same sub-genus Nyssomyia, the Lutzomyia whitmani that is a vector of L. braziliensis, were registered in Jacobina (9,27) and can probably also transmit L. amazonensis.

Didelphis albiventris is the first non canid mammal shown to be naturally infected with L. chagasi (30-33-36) and was also challenged in the laboratory with the same leishmania sp. The histopathologic examination of the specimens naturally infected showed proliferation of macrophages cells but did not reveal parasites or typical evolutive lesions of visceral leishmaniasis. Also, the histopathologic examination of the experimentally infected specimen, with the same opossum strain of L. chagasi, only revealed amastigotes in the spleen and liver and granuloma formation as in the involutive forms of visceral leishmaniasis (23,36).

D. albiventris was captured throughout the year but during the colder months June, July and August the number of specimens collected was higher. This possible variation in the opossum population correlates with the higher density of L. longipalpis and the forthcoming of new human cases of visceral leishmaniasis in Jacobina (33). It is in the colder and rainy months that the opossum frequents human dwellings more often, looking for food. Besides leftovers from human meals, the opossum also eat chickens, eggs and fruit (30).

D. albiventris is the most frequent wild mammal to be found infected with L. chagasi around houses in Jacobina. In two houses close to one where two infected opossums were captured, human and canine cases of visceral leishmaniasis were encountered. Lutzomyia longipalpis feeds readily on D. albiventris as we verified in traps with opossums used as bait in Jacobina, and in the laboratory where the challenged animals infected the sandfly (30).

D. albiventris is an american mammal and does not strongly suffer from infection with L. chagasi. It seems to indicate the existence of an evolved parasite host association that permits this marsupial to be one of the primary natural reservoirs of the parasite of american visceral leishmaniasis.

However, in spite of some evidence, one can not yet eliminate the hypothesis that opossum in its approximation to man, has been infected with leishmanias by the same vector that is infecting man, dogs and other reservoirs.

Foxes - *Lycalopex vetulus* can be seen, during the night, crossing roads and are sometimes killed by cars. It is not easy to catch foxes and for this reason on 14 specimens bought from local hunters were examined. All the specimens were apparently healthy and were not positive for leishmanias. It is impossible to make conclusions about the role of these canids in the epidemiology of visceral leishmaniasis in Jacobina. More studies must be carried out on the foxes.

V. Epidemic Periodicity of American Visceral Leishmaniasis

Based on (i) observations in Jacobina, where the senior author has been working since 1959 (27,28,29,31), (ii) a bibliographic review of the registration of human cases of visceral leishmaniasis in Brazil (2,3,7,8,15,18,19,21,39,40,42) and (iii) on data of Ministry of Health in Brazil (38), it was possible to conclude that visceral leishmaniasis has been occurring endemically with cyclic decenal epidemics. Every epidemic lasts for three years, after which the number of cases begins to decline until they reach the endemic condition. Generally, the peaks of the epidemics have taken place in years ending with the number three and years before and after the principal year, as explained as follows. Beginning from 1913, when Migone (15) described the first human case on the american continent, the highest number of cases were reported in the scientific literature, principally in the years 1923, 1933, 1943, 1953, 1963, 1973 and 1983 (Table XII). The next epidemic is expected to occur in the years 1992, 1993 and 1994 (31).

It is not yet possible to know the real causes of the periodic epidemic cycle. It seems possible that there exists some correlation between the immunological and nutritional status of individuals between 0-5 years of age that are exposed to the disease. We know that malnourished children from the ages 0-5 years old are more frequently suffer visceral leishmaniasis in Brazil (4,5). The serological positive rates of infection predominates in children above 5 years old as noted by Badaro (4,5). Climatic factors and ecological unbalances probably also have a connection to the epidemics. Droughts that provoke a successive chain of interrelative factors such as farming devastation, scarcity of food and decrease of the nutritional and immunological condition of the population challenged. In drought periods the sylvatic reservoir fauna encroach on the peridomestic environment looking for food or shelter which facilitates the transmission of the disease. The periodic occurrence of visceral leishmaniasis is very important to direct more correct and economic control measures at the right time.

TABLE XI
CASES OF VISCERAL LEISHMANIASIS REPORTED TO SUCAM FROM 1976
TO 1983 (FROM THE REPORT OF THE FIRST MEETING ABOUT RESEARCH
ON LEISHMANIASIS, S.PAULO, 1984

Y E A R	N U M B E R O F C A S E S	% T O T A L
1976	95	3.1
1977	57	1.8
1978	86	2.8
1979	91	3.0
1980	165	5.3
1981	359	11.6
1982	1,120	36.3
1983	1,124	36.3
T O T A L	3,097	100%

TABLE XII
CASES OF VISCERAL LEISHMANIASIS REPORTED IN BRAZIL BY SEVERAL
AUTHORS (1913-1986)

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Impr. Univ. Recife
1934 - PENNA, H.A. - Brasil Medico Rio de Janeiro, 11:1-5
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15:97-102
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1954\77 TEIXEIRA, R. - Tese Fac.Med.-Univ.Fed.Bhia, 1980
1973 - SUCAM, M.S. - I Reunião Nac.Pesq.Leishmaniose,SP,1984
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1982\86 SHERLOCK, I.A. et al. - Dados inéditos ref. Bahia

VI. Conclusions

Several papers, principally those of Deane (9) have established the most important geographical and climatic characteristics of the ecology of visceral leishmaniasis in Brazil, its principal vector, and dogs and foxes as domestic and sylvatic hosts, respectively. For the first time, we have recently found the marsupial opossum Didelphis albiventris, naturally infected with Leishmania chagasi, which was confirmed in foci in Colombia (30,42). However, several aspects of the ecology of visceral leishmaniasis still remain unknown making the understanding and resolution of the problem for control difficult.

Several aspects are still not clear. First Chagas et al. (8), and more recently other authors, principally Lainson (13), defend the autochthonicity of Leishmania chagasi to be the etiologic agent of leishmaniasis in America continent. According to this author, if L. infantum from Europe had been introduced in the american continent, human leishmaniasis could not have dispersed so quickly throughout South America, because in the actual areas of distribution of the disease, the means of dispersion were very deficient. So, those authors speculate that dogs, the presumable primitive hosts of viscerotropic leishmanias, were already well established in America, before the arrival of the white man. Thus, there were no reasons for these animals not to have their own leishmaniasis (13).

Truly, one does not know if the american dogs had their own leishmanias in the pre-colombian period. In the same way, at least in Brazil, one does not have any evidence that the natives suffered from visceral leishmaniasis (27). On the contrary, it is certain that in the pre-colombian period, the american indians suffered from cutaneous leishmaniasis (20).

However, as an example of the introduction of Leishmania infantum in the America, Lainson (13) has pointed to the recent establishment of a small foci of canine leishmaniasis in Oklahoma, USA, involving, at least, four dogs outside of the local area. Canine leishmaniasis was also reported seven times in dogs that came from Greece. This evidence suggested that imported dogs were probably the source of origin for the Oklahoma focus. Since the only species of sandfly (Lutzomyia diabolica) is rare in Oklahoma, one can speculate that congenital transmission could have occurred. Later, in Oklahoma it was proven that leishmaniasis was transmitted among dogs via ticks (16).

Most of the leishmanias produce mild or asymptomatic infection in natural hosts, maintaining the balanced relationship between parasite and host. Commonly dogs and foxes have a fulminating infection, thus they are not well adapted to serve as a reservoir for the parasite. On the contrary, like man, they seem to be recent hosts that suffer from the disease. This fact, according to Lainson (13), has been used as an argument for the support of the hypothesis that visceral leishmaniasis was caused by the imported L. infantum. From an epidemiological point of view, regardless of whether or not dogs

suffer from visceral leishmaniasis, they are the principal domestic reservoirs. However, another primary wild reservoir must also exist in order to explain some aspects apparent in the natural history of the disease.

The four first specimens of foxes found naturally infected in America by Deane (9) were sick, but the three other foxes found naturally infected by Lainson and Shaw (13) were apparently healthy. For this reason, those authors think that if a more significant number of infected foxes without the symptoms of the disease were found, one could explain that american visceral leishmaniasis could have originated from old american sylvatic cycles involving foxes and their commensals (13).

In Jacobina the examination of only 14 specimens of foxes with negative results, did not permit any conclusions (Table X). It is suggested that more specimens should be examined (35).

The domestic rodents Rattus rattus and Rattus norvegicus have been recently found infected with L. infantum in Mediterranean areas (10, 22). Alencar (1) found amastigotes in R. rattus in the State of Ceará, Brazil. Lainson and Shaw (13) believe that the parasite could be L. chagasi. The finding of infected rodents with leishmanias in Central America, is believed by Lainson (13) also to be L. chagasi.

Some years ago, the senior author of the present paper, made the hypothesis that the domestic rodents in Jacobina could be the hosts of L. chagasi (29). Unfortunately, like the foxes, the number of rodents examined was very small and thus, no conclusions can be drawn.

According to Lainson (13) serologic studies and isoenzymes electrophoresis do not have definite value in distinguishing L. infantum and L. chagasi. It was possible for him to find differences among the proteic profiles of several leishmanias, which was confirmed by means of polyclonal and monoclonal antibodies with surface antigens of three species. However, it was observed that some close relation existed among L. chagasi from Brazil (13), L. infantum from the Mediterranean and L. donovani from India. Nevertheless Momem, Grimaldi Jr. and Deane (17) did not find differences in the proteic profile using 11 enzymes in 100 isolates of L. chagasi from Brazil (17). They concluded that the profiles of L. chagasi was identical to those profiles of L. infantum standardized by WHO. Thus, they proposed the designation of L. infantum also for the species that cause american visceral leishmaniasis (17). Later, Grimaldi et al. concluded the species L. infantum for Mediterranean visceral leishmaniasis and L. chagasi for american visceral leishmaniasis (11) and this is now the general consenses of several authors.

Studies of Ward et al. (44) confirmed Mangabeira's observations about the morphological differences in the vector L. longipalpis. They detected different compositions of pheromones in accordance with the morphological type, suggesting that

one type is more antropophilic than the other. This fact could explain the existence of areas with sporadic disease and other areas with epidemic disease. Transmission is made by the different morphological types respectively. Despite the morphological variations, the sandfly that is designated as L. longipalpis is surely the principal vector of visceral leishmania in the epidemic, endemic, and sporadic foci (1,9,34).

Relative to inherent factors of the vector, we emphasize some recent observations published in specialized literature. Enzymes, like trypsin, inhibit the sandfly infection with some species of leishmanias (24). Such enzymes are self produced in the digestive tract of the sandfly and their production stimulates the presence of certain species of leishmanias (24). This corresponds to a natural protective mechanism of the sandfly against infection, probably prejudicial for itself. Some investigators discovered that the inhibition of the action of the tripsin enzyme is made by the same substances that inhibit soybean tripsin. When it is ingested by a sandfly an infection with leishmania easy (24). We know that sandflies feed on vegetation both in nature and in the laboratory. In the laboratory, sandflies feed on afidiulutein produced by aphids, which contains several aminoacids and sugars, important in the metabolism of the trypanosomatids. In the laboratory, sugars facilitate the infection of sandflies and increase the number of promastigotes (24).

The type of host seems to be important for improving the infection of the sandfly. Bird blood diminishes the capacity of infection in the sandfly, probably because it contains enyzmes like tripsine.

Recently Titus and Ribeiro (9) discovered an acentuated increase for the infectivity of Leishmania major when inoculated together with salivar glands of L. longipalpis in Balb/C mice.

In spite of the recent findings mentioned above, we were unable to make a sucessful experimental transmission of L. chagasi by means of the bite of L. longipalpis (Table IV). We do not know if the above mentioned factors are interfering in the results of our experiments.

At the present time it is possible to control visceral leishmaniasis at a temporary level through the treatment of human cases, elimination of sick dogs and using insecticides to control vectors. However, it is not possible to obtain permanent control of the disease, since as soon as expensive prophylactic measures are interrupted, the disease continues its natural course. Basic questions still remain without answers such as: Does a natural primary reservoir exist? What are the real roles of man, dogs, foxes, opossums and rats as reservoirs of visceral leishmaniasis? Where are the natural breeding places of the vectors? etc.

Implications for Control

From the point of view of the control of american visceral leishmaniasis the contributions and conclusions of our project are as follows:

- a) We still do not have sufficient knowledge to include fox and opossum reservoirs in control strategies. It appears that dogs are a more important domestic reservoir than man.
- b) Control strategies must include three principle measures: treatment of human cases, elimination of infected dogs, and the use of insecticides to control vectors. The active use of these measures can result in temporary control of the disease.
- c) However, no one measure in isolation is sufficient to produce an acceptable level of control. As has been emphasized before, treatment of human cases together with the killing of sick dogs is not enough to prevent the occurrence of new human cases in Jacobina.
- d) Routine spraying with insecticides every six months when the density of sandflies high, is necessary to control the vector.
- e) Health and sanitation education is necessary for communities to avoid the migration of infected humans and dogs from endemic to indene areas.
- f) Understanding of the cyclic decennial periodicity in the natural histroy of american visceral leishmaniasis is important to maximize the effectiveness and economy of control measures.
- g) Further multidisciplinary studies are necessary to understand how permanent and cost-effective control of visceral leishmaniasis can be achieved.

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Clinical and Field Epidemiological Investigations of Kala-azar (Visceral Leishmaniasis) in West Pokot District of Kenya

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Introduction

During the last few decades, research on kala-azar in Kenya has revealed that the disease is endemic in several arid and semi-arid areas of the country. In order to further understand the epidemiological factors whose interactions result in the creation and maintenance of such foci, a field study was initiated in West Pokot district of Kenya in October, 1985 and successfully completed in March 1988.

The main objectives of the study of were:

- To determine the prevalence of kala-azar in the human population in West Pokot district.
- To undertake ecological studies on the local sandfly species within an established kala-azar focus with particular reference to species composition, seasonal distribution, abundance in different habitats and to investigate their parasite infection rates.
- To investigate the vertebrate reservoirs of kala-azar from a range of local, wild and domestic animals.

Since malaria is also present in West Pokot, a prevalence survey of the disease was carried out parallel to that of visceral leishmaniasis.

Materials and Methods

A) Clinical Survey

Study population: The West Pokot district (35° 15' E, 10° 45' N) is situated in western Kenya along the border with Uganda (Fig. 1). Most of the land lies between 1000 m and 1500 m above sea level. A small area on the south eastern boundary covers part of the

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malaria & leishmaniasis prevalence

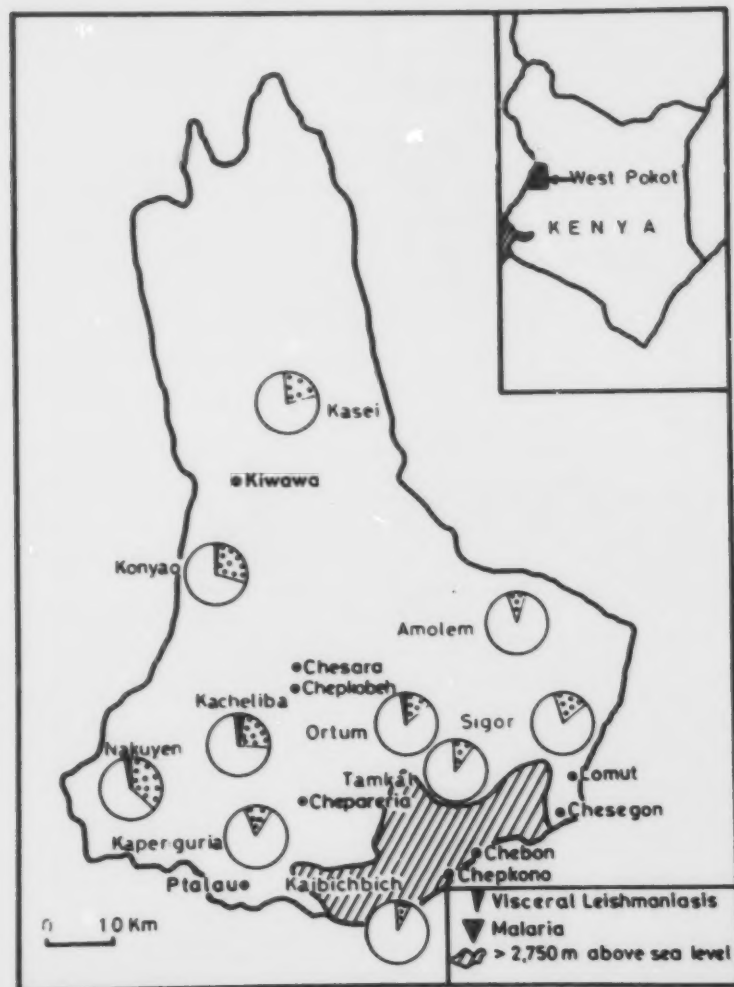


Figure 1: West Pokot District, showing location within Kenya and the proportion of people with Serologically Active Visceral Leishmaniasis and malaria parasites at different screening centres.

Cherangani hills which rise to more than 3000 m above sea level. The vegetation cover comprises acacia woodland and bushland in the lower-lying areas and forests at high altitudes. The mean annual rainfall ranges between 500 mm and 1300 mm. A large portion of the low-lying land is of marginal agricultural potential and the majority of the people are pastoralists. Cultivation of crops is also practised at around 1500m above sea level.

West Pokot has an estimated total population of 210,000 people distributed within 67 administrative sub-locations (Data obtained from the District office). According to the data from the District Office, proportions in the age groups 0-4, 5-14, 15-44, and >45 were 18.5%, 29.8%, 39.8% and 11.9% respectively. About 1% of the population, was used in the epidemiological survey. The sample was derived from nineteen sub-locations (clusters) selected at random. Individuals for screening were systematically selected from a queue at each screening centre. The number of people screened in various sublocations ranged from 50 to 200, in proportion to the relative population of each. In each sub-location, the number screened in each age-group was also proportional to the age group's representation in the whole population. Male and female persons were assumed to occur in equal proportions in the population. The number of each sex included in the screening sample was therefore approximately the same.

Diagnostic methods:

- (a) Spleen examination: All persons being screened had their spleens physically checked by either of two techniques described by Bruce-Chwatt (1985). The spleen rate (percentage showing spleen enlargement) was calculated for each sub-location.
- (b) Blood films: Thick and thin blood films were prepared for each person using methods described by Bruce-Chwatt (1985). The films were examined under oil immersion to identify malaria parasite species and determine malaria parasite rates.
- (c) ELISA: The procedure for ELISA was as described by Ho et al. (1983). During the field survey, each person had one finger pricked with a sterile lancet and 70 μ l of blood collected in a heparinized capillary tube. The blood was emptied within a circle of 1 cm in diameter drawn on Whatman No. 3 filter paper. Individual blood samples on filter paper were soaked in 0.5 ml distilled water at 40°C overnight and eluted to provide test sera. The sera were reacted in micro-ELISA plates against antigen prepared from Leishmania donovani previously isolated from a Kenyan kala-azar patient. A positive reaction corresponding to an active case of kala-azar was indicated by a vividly yellow colour with an absorbance value greater than 0.45 at 492 nm. Borderline cases representative of exposure but not the active disease had absorbances between 0.25 and 0.45 while negative cases were consistently below an absorbance of 0.25.

The proportion of reactions corresponding to active disease was expressed as a percentage to provide the kala-azar prevalence rate. Borderline reactions represented

the proportion of people expressing Leishmania antibodies but not having active disease. Active and borderline reactions combined provided the Leishmania antibody prevalence rate.

(d) Leishmanin test: Antigen for the Leishmanin test (Peters and Killick-Kendrick 1987) was prepared from a Kenyan L. donovani strain by Kenya Medical Research Institute (KEMRI) at a concentration of 2×10^6 organisms per ml. The antigen was reconstituted in 0.5% sterile phenyl saline solution. For each person, one forearm was swabbed with 70% alcohol and injected intradermally with a 0.1 ml dose of the antigen. The other forearm was injected with a 0.1 ml control dose consisting of phenyl saline alone. The reaction on the fore arm was examined after 48 hours and the diameter of the swelling measured in millimetres.

(e) Statistical analysis: Chi-square with Yates correction (Bailey 1981) was used in 2×2 contingency tables to test the difference in disease prevalence between different sexes and age groups. Chi-square was also used in comparing infection rates obtained by various diagnostic methods.

B. Entomological Studies

Sandfly sampling and dissection for Leishmania parasites: On the basis of results from the clinical survey, Kacheliba was selected as an ideal locality for both entomological and reservoir studies. Sandfly sampling was carried out in three different sites around Kacheliba, namely, Sangakai, Kongelai, and Pole. In each site, sandflies were collected weekly from a variety of habitats including houses, termite hills, rodent burrows, tree holes, cow and goat enclosures. Sampling was done using a standard 1 m² sticky polythene trap which was set up at the sites at 1800 hours and removed at 0600 hours the following day. Two such traps were placed in each habitat.

Flies removed from the various traps were washed in water with a little detergent added to it. A subsample was dissected and the gut area examined for Leishmania parasites. The undissected sample including the heads of those dissected were mounted on glass slides for identification into species. Positive guts were inoculated into NNN culture media and taken to Nairobi for further parasite characterization.

Isolation of Leishmania parasites from domestic and wild animals:

Spleen and liver tissue for parasite examination in domestic animals were regularly collected from the local butcher. Small pieces of the organs were homogenized in the field laboratory and inoculated in NNN culture media. Impression smears from the organs were also made on glass slides for the direct examination of Leishmania amastigotes. Media bottles and specimen slides were then transported to Nairobi for examination and further observation on parasite growth.

Results

A summary of results on the clinical survey is presented in Table 1. There was no correlation between the distribution of people reacting positively to the Leishmanin skin test and those having antibodies against Leishmania as demonstrated by ELISA. However, a highly significant positive correlation ($P > 0.01$) was found between the distribution of enlarged spleens and the presence of either Leishmania antigens or malaria parasites.

According to Table 1 and Fig. 1, the proportion of people showing antibody levels that were indicative of active kala-azar ranged from zero to 5% in the various sub locations. The overall proportion of people showing either borderline positive reactions or active disease was much higher and ranged from zero to 20%. The highest incidence of active cases in a screening centre was 5% recorded in Kacheliba.

Presence of Leishmania antibodies was more common in low lying areas around Kacheliba than in the higher altitudes at Kaibichbich. The occurrence of active disease was even more limited to lower areas and was virtually absent beyond 2,000 metres above sea level. The distribution of kala-azar active cases and antibodies showed the incidence to be lowest in the age groups below 4 years and also above 45 years. It was highest in the age group 5-14 years.

The prevalence rate of malaria parasites was higher than that of Leishmania antibodies and also more widespread in the district (Fig. 1). A maximum parasite rate of 32.7% was recorded at Nakuyen, a few kilometres from Kacheliba (Table 1 and Fig. 1). Infection rates were highest in the younger age groups but steadily declined with age. Over 95% of malaria parasites belonged to Plasmodium falciparum, the rest being P. malariae and to a less extent P. ovale. Only one case of mixed infection of P. falciparum in association with P. malariae was recorded.

With regards to entomological investigations, a total of 11 sandfly species was recorded from all the three sampling sites. The most common species were Sergentomyia antenauatus, S. bedfordi and S. schwetzi. P. martini occurred in relatively lower numbers and was most common in termite hill and animal burrow habitats (Table 2). The termite hill habitat yielded the highest number of sandflies in Kongelai. Sangakai had the highest number of sandflies collected around houses while Pole had the lowest. Most of the sandflies in Pole were collected near animal burrows.

The monthly fluctuations in relative density of various species showed a major peak in numbers in January, followed by a steady decline until June when the lowest density was recorded. This population trend was most evident in the termite hill and house habitats in Sangakai. The peak sandfly season coincided with the dry period while the lowest numbers were collected a month after the rainfall peak in May.

Table 1. Summary of results from the clinical survey of West Pokot, 1986

Cluster	No. examined	Leishmanin test % positive	Enlarged spleen %	E L I S A (Visceral leishmaniasis) % active % antibodies	Malaria % positive
Kacheliba	158	0.6	60.8	5.1	22.9
Nakuyen	101	7.9	22.8	3.0	32.7
Konyao	150	2.0	16.7	0.7	23.5
Kasei	50	2.0	2.0	0.0	15.6
Kapenguria	101	1.0	5.0	0.0	15.8
Kaibichbich *	50	0.0	0.0	0.0	4.0
Chepkono *	125	0.0	0.0	0.0	0.0
Ptalau	200	0.5	0.0	0.0	2.1
Chebon *	65	---	0.0	0.0	1.5
Chepareria	126	0.8	4.8	0.8	4.2
Ortum	142	3.5	5.6	1.4	11.3
Chepkobeh	87	6.9	26.4	3.4	6.9
Chesara	124	3.2	2.4	0.8	13.8
Lomut	103	4.9	17.5	1.0	13.6
Sigor	96	7.3	15.6	0.0	20.8
Tamkal	100	13.0	1.0	0.0	7.0
Chesegon	99	5.1	9.1	1.0	4.0
Amolem	125	12.0	12.8	0.0	9.1
Kiwawa	137	---	16.8	1.5	12.0
Total	2139	4.2	11.5	1.0	11.6

* Screening centre lying more than 2750 m above sea level.
 --- Screening not done.

Table 2: Female *P. martini* collected from various habitats
between January and December 1987

Locality	Housewall (Inside)		Housewall (outside)		Treehole	Termite Hill	Rodent burrow	Cow enclosure	Goat pen
Kongelai	No.	0	0	0	2	10	-	-	-
	%	0	0	0	16.6	83.4	-	-	-
Sangakai	No.	1	3	3	2	19	8	-	-
	%	3	9	9	6.1	57.6	24.2	-	-
Pole	No.	2	5	5	-	46	157	9	5
	%	0.9	2.2	2.2	-	20.5	70.1	4	2.2

- Sampling not done in the habitat

The relative density of flies collected inside and outside houses varied between the three sites during different months. However, generally more flies were collected inside houses than in traps placed next to the walls outside, as shown for all the three different sites in Figures 3-5.

Infection of phlebotomine sandflies with Leishmania parasites was observed in February and March, 1987. The overall total infection rate was 1.2%. The parasites were obtained from S. antennatus, S. bedfordi, Phlebotomus martini and S. schwetzi. Identification of an isolate from P. martini which grew in NNN medium is pending.

The processing of spleen and liver material from domestic goats was carried out throughout 1987 in order to investigate animal reservoirs. During the period, a total of nine goats, one sheep, two lizards and one rat were found infected with Leishmania parasites. The parasites from goat and sheep have been tentatively identified as L. donovani and L. major using isoenzymes.

Discussion

Several observations were made during the study which may be of major implications in leishmaniasis epidemiology in the East African region. Results on prevalence showed that within the West Pokot focus, the disease existed in several geographically defined micro-foci of varying severity. The disease was also found to be limited by altitude as was clearly indicated by an absence of anti-Leishmania antibodies in people living in high altitudes. Although familial clustering of leishmaniasis has been reported by other workers in the past (Mutinga et al. 1984) the present extensive study provides useful information on the geographical distribution of the disease. This knowledge will be particularly useful in the proper direction of disease control measures to those areas where they are most urgently needed.

In addition to prevalence of leishmaniasis, this study yielded important data on the occurrence of malaria in West Pokot district. Malaria infection rates were generally higher than those of leishmaniasis. However, the ranges of these two diseases were found to overlap considerably. A significant positive correlation of the distribution of either disease with that of splenomegaly was established, and the occurrence of enlarged spleens alone was therefore considered an inconclusive method for screening the diseases. Nevertheless, the results on the distribution of the two diseases underlined the importance of a multi-disease systems approach in future studies and during the implementation of disease control measures. This is in order to ensure that the resources available for research and improvement of the health of the affected populations are most effectively and economically utilized.

With regard to studies on animal reservoirs, the occurrence of Leishmania parasites in goats and sheep spleen and liver and in skin lesions was particularly

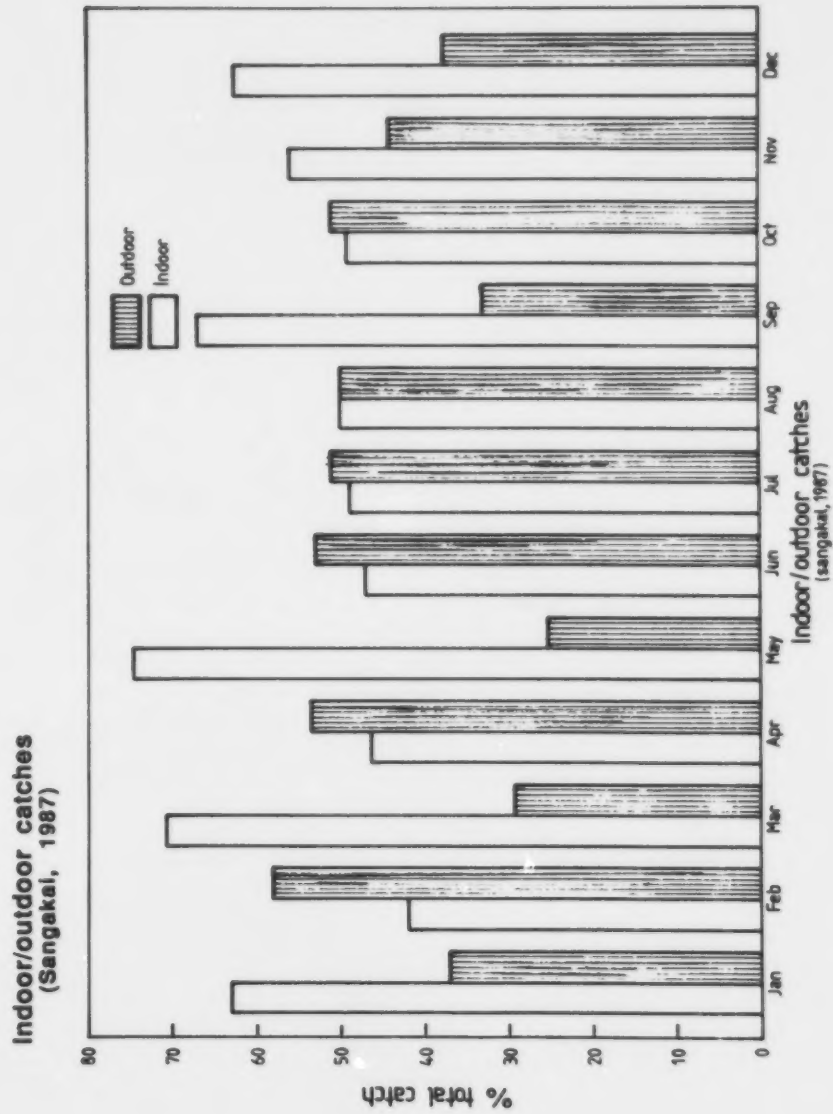


Figure 3: Proportion of sandflies collected outdoors and indoors in Sangakal.

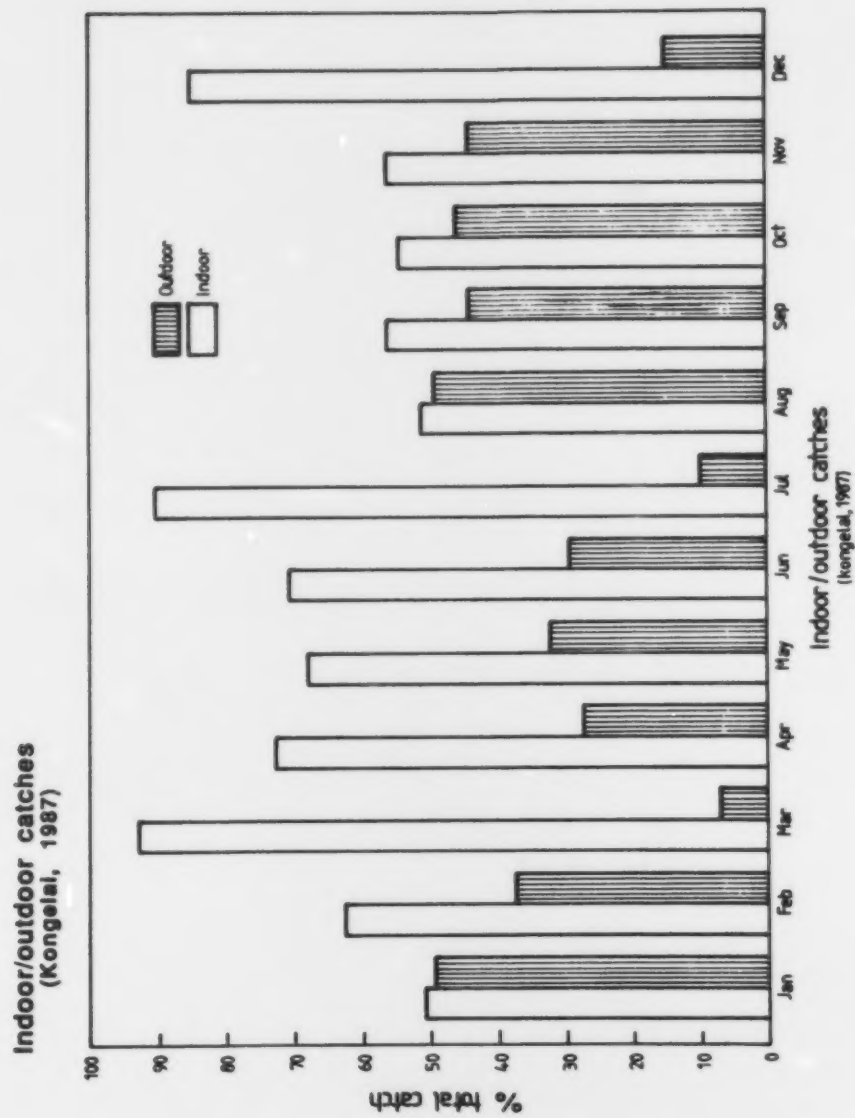


Figure 4: Proportion of sandflies collected outdoors and indoors in Kongelai.

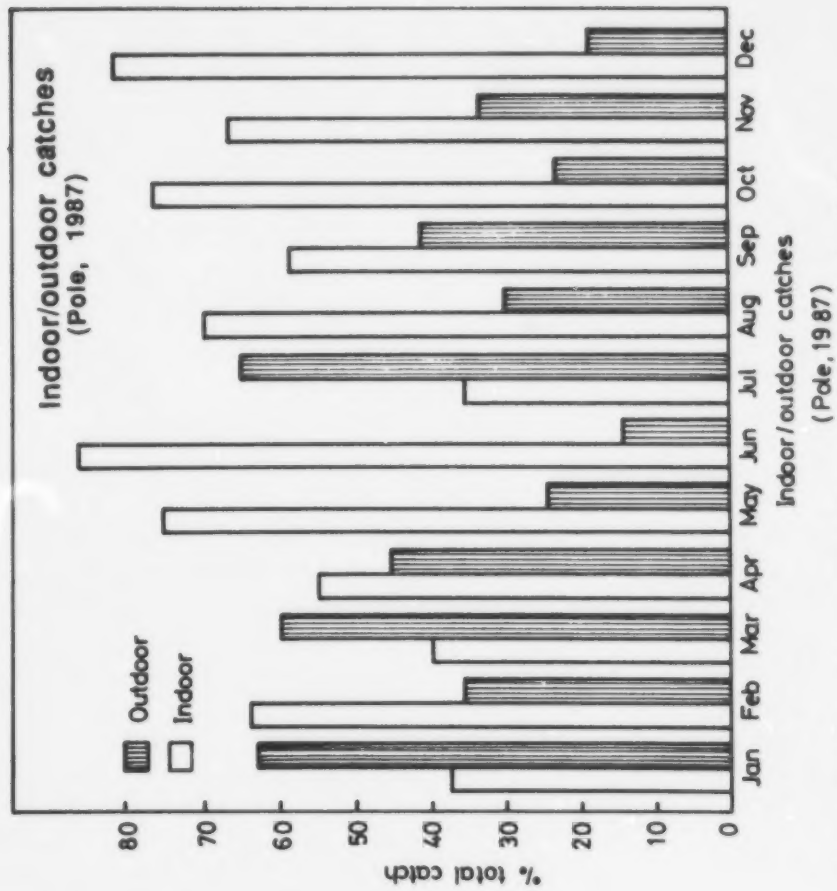


Figure 5: Proportion of sandflies collected outdoors and indoors in Pole.

significant. Workers in Africa and elsewhere have in the past shown very little interest in the possibility of domestic animals being reservoirs of leishmaniasis. However, our finding of several infected goats and sheep from within such a small area might suggest that these animals could be reservoirs in the various leishmaniasis foci in the East African region. This observation is especially important in view of the close association that normally exists between people and their livestock in most kala-azar areas. Tentative characterization reveal that the goat and sheep leishmania are similar to those that affect man, i.e. L. donovani and L. major.

Results on phlebotomine sandfly studies revealed valuable information regarding their distribution in various habitats during different times of the year. Termite hills and rodent burrows were particularly the favoured concentration sites for the various species. Occurrence of flies in large numbers inside houses suggests probable larval breeding indoors; a phenomenon that has been observed in Marigat area of Kenya (Mutinga, et al. 1989). It is also worth noting that S. antennatus, in addition to being the most common species, also yielded several females with Leishmania parasites. Although Sergentomyia antennatus has not in the past been implicated in the transmission of kala-azar, its possible role requires further investigations both in the field and in the laboratory, in view of recent findings that S. garnhami and S. ingrami are probable vectors of mammalian Leishmania (Mutinga and Odhiambo 1982; Mutinga et al. 1986).

With the establishment of the resting and breeding sites of sandflies in major endemic foci of leishmaniasis in Kenya, ICIPE is now well set to embark on a pilot control scheme of sandfly and mosquito populations. It will be possible to target integrated control measures inside houses, around termite hills and animal burrows appropriately as well as breeding places for mosquitoes. Inspired by the simplicity and low cost of the "ICIPE Sticky trap" (Mutinga 1981) for the control of sandflies, made of polythene sheets and smeared with castor oil, the Medical Vectors Research Programme has developed the "MBU Cloth"⁴, a simple cloth made from cotton and hung on the wall and impregnated with permethrin insecticide at dosage of 0.5g per m². The cloth is hung along the wall of every dwelling of rural house; it is of appropriate size worked out to ensure elimination of female mosquitoes and sandflies before they are able to acquire infection. This technology has been tried by ICIPE on a small scale and has shown very promising results.

It now requires a larger scale trial with very well controlled scientific data gathering to provide information on the prevalence of vector and parasite populations before, during and after the intervention measures. The merits of this new control strategy are:

- (a) The cloth is simple and is very easily accepted by the local population. For rural

⁴ MBU is a swahili word meaning mosquito.

areas where beds are rarely used, a single wall cloth can protect an entire family sleeping in the same house. The effect of the cloth is apparent almost immediately therefore its usefulness is appreciated quickly by the people.

- (b) It is made of locally available raw material, i.e. cotton, and hence it is affordable. Approximately 9 m² of the cheapest cotton cloth or material is required and lasts over 4 years.
- (c) The permethrin insecticide used is safe and limited in application only to the cloth which hangs on the wall away from children and adults. The impregnated cloth controls a variety of vectors which frequent dwellings such as mosquitoes, fleas and a variety of houseflies.
- (d) The insecticide is long-lasting (lasts for 6 months) and the cost of impregnating each house is approximately US\$ 0.80.

This new innovation we feel, is an additional new tool to existing ones in an attempt to control leishmaniasis and malaria vectors inside houses. For outdoor populations, we still have to continue to look for alternative control measures such as biological control agents, i.e. bacteria and other protozoa and fish.

For leishmaniasis, the question of animal reservoirs both wild and domestic remains still a very crucial issue. New findings strongly suggest the involvement of animals that have previously been least suspected, e.g. domestic goats and sheep. This area needs urgent attention, in our view, in order to know whether or not these very close associates of man contribute to the dissemination of leishmaniasis or whether they are mere accidental hosts without threat to health.

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Migration, Settlement Patterns and Visceral Leishmaniasis in Ethiopia

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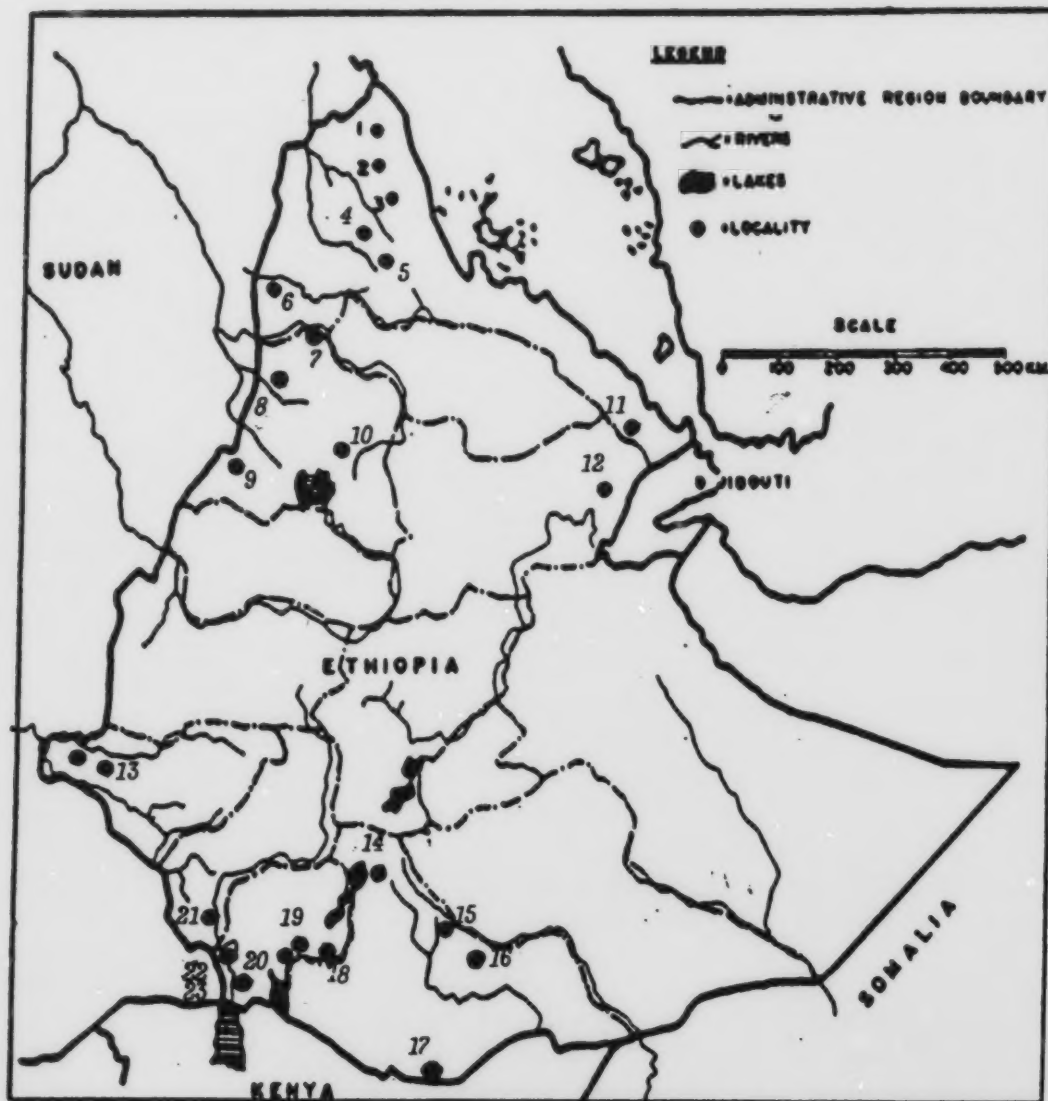
Introduction

The control of leishmaniasis in Ethiopia remains a major challenge for many reasons. Research and training towards the establishment of a national leishmaniasis control programme is only at its early stage while leishmaniasis is increasing alarmingly vis-a-vis the identification of new foci and increased population movement into and out of endemic areas. Population movements result from resettlement schemes, large-scale agricultural development and the displacement of humans within or from neighbouring countries like the Sudan and Somalia as a result of war, drought and famine. In Ethiopia, leishmaniasis has spatially varied disease ecologies and each ecological niche need to be dealt with separately. To mention a few, the highland CL associated with rock hyraxes, the anthroponotic type VL associated with termite-mounds and the zoonotic type VL associated with deep soil cracks of Acacia-Balanites woodland are prominent categories. Very little is known about lowland CL associated with *L. major*. Whereas this diversity is a major challenge, it presents a treasure of research opportunities and practical experiences that need to be exploited.

Leishmaniasis in Ethiopia is caused by *L. donovani*, *L. aethiopica* and *L. major*, the later two causing cutaneous leishmaniasis (CL). *L. donovani* causes visceral leishmaniasis (VL) and is largely restricted to low altitudes (< 1300 m.a.s.l.) in the arid and semi-arid parts of the country. The western littoral of the Red Sea (Algena, Nakfa, Afabet), the western lowlands of Eritria (Teseney), the Metema-Humera plains, the lowlands of SW Ethiopia (Segen, Woito and lower Omo plains) and Gelana, Wadera, Negele and Moyale in the south are known to be endemic areas of VL (Fig. 1). These areas are mostly inaccessible and have very limited health services. The magnitude of VL in these areas therefore has not been determined except in one focus -- the Aba Roba focus in the Segen river basin of Ethiopia. Similarly, little is known about the vector, reservoir hosts and transmission pattern in most foci. In many of these foci, other tropical diseases are co-endemic. Areas in the west, south and southwest are located in frontiers with VL endemic areas from north Kenya, southeast and eastern Sudan. Endemicity was determined by gathering together information from a few entomological studies, active case findings and leishmanin skin test surveys.

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Figure 1: Map showing geographical distribution of visceral leishmaniasis cases in Ethiopia.



- 1) Algona; 2) Nakfa; 3) Afabet; 4) Asmat; 5) Halhal; 6) Tesenay; 7) Umhajer;
 8) Humera; 9) Metema; 10) Belesa; 11) Chibdo; 12) Mille; 13) Gambela;
 14) Gelana; 15) Negele; 16) Wadera; 17) Moyale; 18) Aba Roba; 19) Kolme;
 20) Wotitu; 21) Kangatan; 22) Kibbish; 23) Omarate

Study areas: 13, 14, 18, 19, 20, 21, 22, 23

Ferro-Luzzi (1) reported 9 cases in Eritrea. Coles and Cosgrove (2) documented VL cases from areas near Lake Turkana. Anderson (3) reported 136 cases in two African battalions that patrolled areas south and southwest Ethiopia, north and northwest Kenya and southeastern Sudan. Another 50 to 60 cases of VL are also known to have been recorded from Kibbish and Omorate - areas in lower Omo in southwest Ethiopia - in the years 1926, 1941 and 1961 (4).

In the northwest, Tekle et al. (5) described a few cases from Setit-Humera; and later, Mengesha and Abuhay (6) and Maru (7) described 27 and 18 hospital cases respectively. Ashford et al. (8) also presented a few locally acquired cases in the northwest, at a place called Belessa (1800 m.a.s.l.). Fuller et al. (9, 10) conducted a survey in the Omo and Akobo river basins (southwestern Ethiopia) and found a few cases. Not much is known since then. In a recent survey made in 1991, two locally acquired cases of VL have been identified in Kibbish (Institute of Pathobiology (IPB) data bank, unpublished). Lintdjorn (11) and later Lintdjorn and Olafsson (12) reported 35 cases in Sidamo and Gamo Gofa administrative regions respectively, mainly from among the Konso people in the Segen and Woito river basins. These reports prompted the staff of IPB to conduct a long term epidemiological study in one focus -- the Aba Roba focus in Konso.

In this study which is now in progress, a total of 164 cases have been diagnosed between March 1982 and May 1991. Part of these results have been reported by Ayele & Ali (13) together with other cases from different parts of the country (Fig. 1). A total of 297 cases were recorded in the data bank of IPB by May 1991. These cases originated from Sahil, Keren and Teseney in Eritrea, Setit-Humera in Gondar, Mille in Wollo, Ghibdo in Assab, Negele, Wadera, Moyale, Mega and Gelana in Sidamo and Konso, Woito, Kibbish and Omorate in Gamo Gofa administrative region (Fig. 1). The northeastern parts of the country are also considered to be endemic. Schaller and Kuls (14) indicated the occurrence of VL in the Danakil and in areas around Jijiga. In Hararghe, Fuller (15) conducted a skin test and case-finding survey in the middle and lower Awash river valley. No evidence of active VL was provided among 319 individuals screened, though a positive skin test of 60% was noted among the Afars. A similar positive skin test rate (61%) was reported in lower Omo (10) and 30% in the Metema-Humera plains (16). In a recent survey (Hailu et al., unpublished), positivity to leishmanin skin test was noted to be around 63% among the Dyssanetch and Bumes in lower Omo, 22% among the Konso in Aba Roba, 8-11% among the inhabitants of Gelana and also among the Nuers and Anuaks in Gambela. Apparently, there is no concordance between incidence or prevalence rates of overt disease and skin test positivity. In a three-year survey of VL in lower Omo, only 3 cases could be detected while 93 were detected in Aba Roba. Skin test rates were 63% and 22% in lower Omo and Aba Roba respectively. An inverse relationship is possible, herd immunity together with other factors possibly play a part in determining fates of infections. Reactivity to leishmanin could be induced by mammalian as well as reptilian leishmania. The latter are parasites of sandflies in the genus Sergentomyia which might also bite man.

Recent ELISA-based seroepidemiological surveys (Hailu et al. unpublished data) also indicated the following positivity rates, i.e. 17% in lower Omo among the Dyssanetch and Bumes, 6.7% in Aba Roba, 6.3% among Nuers and Anuaks in Gambela, and 4.3% in Gelana. On the basis of leishmanin skin test and serological test results, it can be shown that rates of subclinical infections are much higher than overt disease rates. Five to twenty subclinical cases have been estimated for every symptomatic VL case in countries like Brazil and Kenya (17,18). This figure is about 7 in Aba Roba focus and larger in areas of low incidence e.g. lower Omo (Hailu et al., unpublished). Which factors activate latent VL infection is a moot question, although malnutrition (18,19) is believed to precipitate full-blown VL. Some infections are also believed to resolve spontaneously either after symptomatic or asymptomatic disease states.

The age and sex distribution of VL cases depends on whether data was obtained from hospitals or from active surveillance in endemic areas. Cases reported by Anderson (3), Tekle et al. (5), Mengesha and Abuhay (6) and part of the report by Ayele and Ali (13) are mainly adults. These reports are either based on patients of military origin or farm labourers who were somehow able to self-present to very distant hospitals. On the contrary, Lintdjorn and Olafsson (12) and later Ayele and Ali (13) provided the age and sex distributions of cases as obtained from Aba Roba focus. Patients from this focus originated from wider age range. The age range of 164 patients diagnosed between March 1982 and May 1991 was 1-48 years (15.3 ± 9.9 years), 3-48 years (16.2 ± 10.6 years) in males and 1-36 years (13.4 ± 8.0 years) in females. Ages between 5 and 34 years (5-39 in males and 5-24 in females) were mostly affected although a slight increase was noted in the age interval 10-19 years (10-34 years in males and 5-14 years in females). It occurred in both sexes with preponderance in males 2.0:1, compared with a male to female ratio of the Aba Roba population in 1990 of 1.00:1.02. Females are affected at younger ages when they are equally active outdoors like their male age counterparts. Infants or children under age of 5 years, retired men or adults above age of 50 years and women of child-bearing age (>15 years) are at lesser risk. The latter have limited outdoor activity. Analysis of the age and sex related profiles of leishmanin skin test reactions (from August 1990; Hailu et al., unpublished) also support the above. Adult skin test positivity rate of 33% (59% in males and 19% in females) were attained by the age of 15 years. Overall rates were 22% (31% in males and 14% in females), with 2.3:1 as the ratio of male to female positivity rate.

In particular, children below the age of 5 years, and to a lesser degree those below the age of 14 years had very low positive rates. This particular age specific pattern contrasts with the domestic zoonotic VL in Brazil (18) where it occurs in young children. It also indicates an outdoor exposure to transmission implied in a similar study at Baringo, Kenya. Konso children and adult males herd cattle or farm in the Segen river basin and surrounding lowlands. Adult females are active mainly in the peridomestic surroundings though they may also visit the valley to bring food to their families (13,21) and also work during harvest. In other areas, like in the lower Omo high skin test

positive rates are noted among pastoralist children (Hailu et al., unpublished) also showing a pattern pertinent to life styles of the Dassanetch and the Nyangatom and the particular ecological setup of the disease. Among migrant workers of large-scale farms in northwest Ethiopia skin test positivity paralleled the length of stay by the farmers in the endemic area (16). Where VL occurs sporadically the disease might be distributed in all age groups as is the case with cutaneous leishmaniasis in Sebeta near Addis Ababa (22).

In East Africa P. martini, a species associated with eroded termite hills, has been incriminated or suspected as a vector of VL in almost all endemic areas in Kenya (23,24,25,26,27), in the Aba Roba focus in southwest Ethiopia (28), and in the Kapoeta focus (29) in southeast Sudan. P. orientalis, a sandfly associated with the Acacia-Balanites woodland and deep soil cracks, has either been incriminated or implicated as a vector of VL in most endemic areas of the Sudan and Ethiopia (8,29,30,31,32,33). The presence of a black cotton-clay soil in the Acacia-Balanites woodlands is considered to provide a favorable breeding and resting microhabitat for P. orientalis (10,29,30,33). This species has been collected from areas in the northwest of Ethiopia in Belessa (8), Metema-Humera and Teseney (30,33), from southwest Ethiopia in lower Omo (31) and from Awash valley (32). Human infections from P. orientalis, which bites man readily, are probably extradomestic in the forests (29,30) and sometimes peridomestic (8). Humans, therefore, acquire the infection while intruding into the natural zoonotic cycle. Ashford et al., (8) identified blood meals in P. orientalis originating from wild animals. In the Paloich-Malakal focus, VL is considered zoonotic. Arvicanthus niloticus, Acomys albigena, Rattus rattus, Felis serval and Geneta senegalensis are known to harbor L. donovani (29). Very little is known about reservoir hosts in Ethiopia. Unidentified leishmania have been isolated from Arvicanthus (34). Incrimination of P. orientalis in Ethiopia was based mainly on distributional evidences and its readiness to bite man. Further evidence of natural infections and vectorial capacity are necessary to substantiate this assumption.

The ecology of termite-hill associated VL in Ethiopia has been explored far better than the Acacia-Balanites associated VL. In a recent epidemiological study at the Aba Roba focus, natural L. donovani infection of P. martini has been demonstrated (Gebremichael et al. unpublished; Gemetchu et al. unpublished). P. martini and P. celiae associated with termite hills, bite man readily outdoors in peridomestic surroundings (Teferi et al., unpublished; Gebremichael et al., unpublished). This finding is corroborated by other epidemiological evidences like age and sex related disease rates and leishmanin skin test reactivity profiles that suggest outdoor exposures (Hailu et al., unpublished). Spatio-temporal variations in disease and infection/exposure rates in this focus are density dependent. Presence and absence of termite-hills, seasonal abundance and biting density of P. martini, human population size and settlement patterns are among the few determining factors that affect the extent of man-fly contact. It is considered that specific human activities like tending cattle, scaring animal pests and harvesting, predispose the Konso to sandfly bites. Transmission from man to man plays

an important role in this focus. *L. donovani* infections in domestic or wild animals are yet to be discovered. Although *P. martini* has been recorded from Dire Dawa (35), Belessa (8); and from Kibbish in lower Omo and Gelana (IPB, unpublished), its role in the transmission of VL in these places is as of yet unknown.

Objectives of the On-going IDRC-Supported Study

A socio-epidemiological survey of VL in agricultural development and resettlement schemes was launched beginning September 1988. The central objective of the survey was to study the magnitude of the disease and its socio-economic implications with the view of formulating preventive and control strategies in the various ecological settings of the country.

Methodology

Selected areas in the south, southwest and western Ethiopia where VL is known to occur and where human resettlement has been occurring were selected. The study areas included:

1. The Konso area in the Segen Valley, southwest Ethiopia.
2. The Woito resettlement scheme in southwest Ethiopia
3. Omorate & Kibbish in southwest (an area of agricultural development)
4. Itang (Gambella) in western Ethiopia, and
5. The Gelana resettlement scheme in south Ethiopia.

Repeated cross-sectional surveys were carried out in six foci (i.e. Woito, Kolme, Omorate, Kibbish, Itang and Gelana). A longitudinal study was launched in one focus - the Aba Roba (Fig. 1). The methods employed are as outlined below:

A. Cross-sectional

1. Active case-finding surveys
2. Passive case-finding surveys
3. Serological mass screening
4. Leishmanin skin test surveys
5. Entomological surveys
6. Search for animal reservoirs
7. Socio-demographic surveys

B. Longitudinal

1. Quantitative epidemiological studies
2. Risk factor studies (migration, settlement patterns, age, sex, occupation).

Passive case detection was also carried in hospitals from Addis Ababa where patients are referred. The history of migration was recorded. This information was used to carry out an active case detection survey in areas alleged to be transmission sites. Data on migration was obtained from local government offices, the Relief & Rehabilitation Commission and from the Institute of Development Research of Addis Ababa University. Aerial photographs were also studied to examine dispersion of villages in Konso sub-district. Changing patterns of settlement in Konso were studied during the socio-economic surveys.

Results

I. Population Distribution in Ethiopia

The distribution of the Ethiopian population has been changing gradually for over 100 years along with changing land ownership and land use patterns. However, swift changes in migration and settlement patterns have also occurred episodically with government land reform and resettlement schemes. In 1984, the population in the central and northern highlands comprised 89% of the total, in an area representing only 38% (36). The unbalanced population distribution has resulted in severe land degradation and disequilibrium in human ecology. This has resulted in at least two major famines in 1974/75 and 1984/85. The lowland areas, relatively sparsely populated, have been rendered unproductive. This is partly because of the prevailing tropical diseases of man including malaria, yellow fever, visceral leishmaniasis and schistosomiasis. Down-slope movement is now taking place increasingly, a trend that has brought forth a new avenue in the human ecology vis-a-vis the increasing suffering of the Ethiopian population from the mentioned above diseases.

II. Settlement & migration patterns in Ethiopia

Four settlement patterns were identified:

- 1) Government-sponsored ("large-scale", "low-cost" or "integrated" schemes)
- 2) War-driven
- 3) Spontaneous, and
- 4) Temporary migrations

These migration patterns have resulted in the influx or outflux of large numbers of people to areas endemic for tropical diseases. Tables 1, 2 and 3 provide a summary of major resettlements that have occurred in Ethiopia mainly due to government-sponsored schemes. Data on migration due to war, famines and on temporary migrations are scarce. In the Ethio-Sudanese border, an estimated number of 350,000 Sudanese refugees have been settled since 1983 at Itang refugee camp.

Temporary and spontaneous down-slope migration and settlement is also known to have taken place by the Konso living in southwestern Ethiopia (37). By studying aerial photographs and also from studies carried by Hallpike (38) it has become apparent that the Konso have gradually dispersed from large hill-top villages to smaller low-lying villages in the nearby lowlands. In addition, hill-top villagers of Konso highlands are practising temporary migrations to the lowlands for agricultural purposes.

Table 1: Major Population Movements (Resettlements) in Ethiopia

Period	No. resettled	Nature of Movement	Distance covered
Before 1950	?	Spontaneous	Short (<200 kms)
1950 - 1970	1,000,000	Spontaneous	Short
1974 - 1980	40,000 (55 schemes)	Govt-sponsored	Short + long
1979	110,090 (84 schemes)	Govt-sponsored	Short + long
1984 - 85	600,000 (280 schemes)	Govt-sponsored	Long
1986 - 1988	Less than 10,000	Govt-sponsored	Long

Sources: Kloos & Adugna (36); Pankrust (39); Bedri et al. (40); Brüne (41)

Table 2: Major regions of settler origin and settlements
2A. Percentage of settlers by origin

Region	Percentage	Endemicity for VL
Wollo	61.0	Focal in eastern lowlands
Shoa	18.9	?
Tigray	15.5	?
Gojam	2.9	?
Gondar	1.1	Western lowlands

2B. Percentage of settlers received by region

Receiving region	Percentage	Endemicity
Wollega	50.4	?
Illubabor	24.3	?
Gojam	12.4	?
Keffa	10.3	?
Gondar	1.2	Western Lowlands
Shoa	1.2	?

? = Unknown

Source: Kloos & Adugna (36).

Table 3: Resettlements in VL survey areas

Locality	No. Settled	No. of Villages	Year of settlement	Nature of settlement
Woitu	155	1	1983	-Spontaneous -"low-cost"
Gelana	1200	2	1979-80	-Spontaneous -"integrated"
Gambela	6318	6	1985	- "Large scale" or "low cost"
[Baro Abol]				
[Shebo]*	14,484	9	"	"
[Perbonge]*	6613	6	"	"
[Ukuna]*	2545	4	"	"
[Ubala]*	8934	9	"	"

* = No VL survey done (Source: CSA (42); IPB data (1984 -1990)).

Visceral leishmaniasis field surveys were carried in selected areas represented by the four aforementioned migration and settlement patterns. As VL has not been considered in the planning and the implementation of resettlement schemes, it was necessary to carry a base-line epidemiology of the disease. The areas of survey and the work done is summarized in Tables 4a and 4b.

Table 4a: Summary of Active Surveillance, 1982 - May 1991

		Leishmanin	ELISA		
Locality	Study popn. (# examined)	Number tested (Percent +ve)	Number tested (Percent +ve)	# of VL cases	#of Sandflies
Gambela					
(Itang)	-Refugees (657)	433(34.4)	438(15.8)	8	3333
	-Indigenous (320)	277(7.6)	320(6.3)	12*	
	-Settler & staff (326)	301(1.7)	317(8.5)	0	
				0	
Gelana	-Indigenous (157)	106(6.6)	136(5.1)	4	903
	-Settlers (526)	203(12.8)	203(3.9)		
Lower Omo					
(Kibbish)	-Indigenous (978)	539(56.1)	659(17.1)		3234
(Kangatan)	-	-	-	2	1028
(Omorate)	-Indigenous(1312)	175(74.9)	164(17.1)	1	1256
	-Farm laborers(260)	247(13.4)	223(5.8)	0	
	-Mixed (146)	143(45.5)	116(7.8)	0	
Konso				31**	
(Woitu)	-Settler (178)	-	116(18.1)	1	194
(Kolme)	-Peasants(891)	-	304 (8.7)	15	
(AbaRoba)	-Peasants(3500)	2603(21.9)	2599 (6.7)	164	
(Other PAs)	-Peasants(698)	-	-	32	1446
All Places	9949	5027	5638	270	

* Diagnosed by MSF Holland/ Belgium (1990)

** Diagnosed by Lintdjorn & Olafsson (12)

Table 4b: Summary of Active & Passive Surveillance. Distribution of VL Cases by Region and District, 1982 - May 1991

Region	District (Areas)	No. of cases
Eritrea	- Sahil (Algena, Afabet, Nakfa)	52
	- Keren (Halhal, Asmat)	7
	- Gash & Setit (Teseney)	3
	- Assab (Ghibdo)	1
Gondar	- Wegera (Humera, Umhajer)	2
	- Unknown	1
Wollo	- Awsa (Mille)	1
Illubabor	- Gambella (Itang, Jekawo)	12* + 8
Gamo Gofa	- Konso (Segen, Woito)	31* + 212
	- Kuraz (Omorate, Kibbish)	3
Sidamo	- Gedeo (Gelana)	4
	- Borena (Negele, Wadera)	1
	- Arero (Moyale)	1
Gojam	- Unknown	1
All places		43* + 297 = 340

* Diagnosed by Lintdjorn & Olafsson (12) and by MSF Holland/Belgium 1989

III. Epidemiology of VL

A. Konso areas

This is a subdistrict in north Omo region, southwest Ethiopia comprising 34 Peasant Associations (PAs) that make up the catchment areas of the Segen-river basin. The population of the subdistrict in 1984 was estimated to be 85910 (43) with human density close to 47 persons/km² (CSO,44). The area is found in the altitude ranges of 800-1900m and with a total annual rainfall range of 200-1000 mms which mostly occur in the months of March, April and May. Eighty five percent of the area is either arid or semi-arid. All except the riverine vegetation and the dry open shrub savannah, is under cultivation. The soil in the slopes is poorly developed and the Konso have always depended and learned to conserve soil and to inhabit hill-tops (Fig. 2a). This strategy was adopted not only to optimize the use of land but also to avoid malaria and tribal conflicts with the neighbouring Borena tribesmen (37).

However, the Konso gradually dispersed to down-slopes. The number of settlements has gone from 78 to over 100 in the last 20 years (37,38). Lowland cultivation and animal keeping in Konso lowlands is done peculiarly by temporary migrations. The migration and land use patterns in Konso district has had an important bearing on the epidemiology of VL. In the years between 1982 - May 1991 a total of 243 cases of VL have been detected (12, IPB data, 1984, 1991). The distribution of the 212 cases by PAs is shown in Fig. 2b, the largest number being from Aba Roba. All cases are reported in 13 (of 34) PAs. These PAs are all southerly adjoined to the Segen and Woitu river beds; 164 cases originated from Aba Roba PA which itself is comprised of six villages. Leishmanin reactivity, seroprevalence, and incidence in these villages varies spatially (Table 5). This is related to altitudinally determined density-dependent human, biotic and environmental factors. The disease in this focus is transmitted by P. martini and P. celiae (Gebre-Michael et al., IPB data, unpublished; Gemetchu et al. unpublished). These phlebotomines are associated with eroded termite-hills.

Density-dependent phenomena affecting the degree of man-fly contact include:

- 1) sandfly abundance
- 2) numbers of termite hills in the villages and farms
- 3) numbers of biting flies (biting-density)
- 4) extent of contiguity and separation between houses and termite hills
- 5) extent of interspersing between houses and termite hills.

Figure 2a: Map showing settlements in Konso, SW Ethiopia



Fig. 2b: Map showing the distribution of 212 VL cases in 34 Konso Peasant Associations (PAs), 1982-May 1991.



Note: 1(1), 2(12), 3(164), 4(11), 5(3), 6(1), 9(3), 10(6)
11(5), 12(1), 17(1), 22(3), 33(1), All(212)

Figures in parenthesis are numbers of cases and numbers 1-34 show Peasant Associations, also shown in Fig. 2a.

Table 5a: VL in Aba Roba, Spatial & Altitudinal Distribution by Village

Village	Altitude (m)	# of houses	Total Population (May, 1990)	Total # of cases	No. of termite hills (1991)
Gabo	1590	94	558	9	-
Foro	1480	258	1449	24	-
Kormale	1460	158	936	32	-
Saba	1420	58	429	4	-
Goinada	1250	140	920	40	178
Galga	1200	114	573	55	229
All villages		822	4865	164	822

Table 5b: VL in Aba Roba, Incidence, Prevalence and Exposure Rates

Village	Annual incidence(%0)	Leishmanin (% +ve)	Seropositive (% +ve)
Gabo	2.2	18.6	3.8
Foro	2.0	15.8	4.4
Kormale	2.4	15.1	8.6
Saba	1.0	20.6	4.9
Goinada	5.7	29.2	8.8
Galga	10.3	26.6	6.7
All villages	4.2	21.9	6.7

With decreasing altitudes the extent of man-fly contact is increased, hence, the lowlands around the two villages named Galga and Goinada present a higher risk for exposure and infection in Aba Roba. About 40% of the cases in Aba Roba are from the four hill-top villages. These cases are presumed to have acquired the disease in the lowlands at the 'Foras' and 'Seferas'. 'Foras' and 'Seferas' are open outlying posts located away from Konso hill-top villages towards the Segen river valley where people spend nights usually a week, a fortnight or even months while tending cattle or for other agricultural activities. Inhabitants of Goinada and Galga use the lowlands in the Segen valley more frequently than other members of their PA in Aba Roba. No animal reservoirs are yet discovered in this focus; man to man transmission presumably plays an important role.

B. The Gelana focus

This area was identified as a VL focus in 1985. The indigenous population of this area are the Guji and Koyra. However, in the Gelana river bed about 390 families (total number of 1350 individuals) displaced during the Ethio-Somali war, were resettled in 1979-80. The resettlement scheme was sponsored by the Relief Rehabilitation Commission, Ethiopia. Three villages, two representing the resettlement scheme and one the indigenous population (Table 4a and Table 6) were surveyed on four occasions for active case findings, leishmanin and serological surveys and for entomological studies. Four patients were identified, all adult females aged 25-35 years among the Guji and Koyra ethnic groups. A total of 528 settlers and 157 indigenous inhabitants (Table 4a) were examined. ELISA and skin tests were employed to screen the settler, as well as the indigenous, populations.

Table 6: Summary of Leishmanin & Serological Findings in Gelana

Population	Village	% Positive (Leishmanin)	% Positive (Serology)
Settlers	Kombolcha	11.1	4.7
	Jeldo	17.8	<1.0
	Both	12.8	3.9
Indigenous	Edibira	6.6	5.2
All villages		11.1	4.3

Table 7 shows the total number of sandflies collected on three occasions by various collection methods. *P. martini*, a phlebotomine incriminated as a vector in Aba Roba focus was also collected. Eroded termite hills are also common. This area makes up part of the rift valley east of lake Abaya at an altitude of 1300 metres.

Table 7: Sandfly Species from the Gelana Valley, Oct-Nov; Feb.March, 1985; Oct-Nov, 1990

Species	Method of Collection									
	Sticky		CDC		Aspirator		Human		Total	
	Plate						bait			
	M	F	M	F	M	F	M	F	M	F
<i>S. Squamipleuris</i>	0	0	10	6	0	0	0	0	10	6
<i>S. bedfordi</i>	145	59	14	28	272	70	0	0	431	157
<i>S. africanna</i>	134	68	3	4	2	3	0		139	75
<i>S. adleri</i>	1	0	0	0	0	0	0	0	1	0
<i>S. heisch</i>	0	1	0	0	0	0	0	0	0	1
<i>S. clydei</i>	0	0	1	0	0	0	0	0	1	0
<i>S. antennata</i>	3	8	0	0	0	0	0	0	3	8
<i>S. schwetzi</i>	6	0	6	0	10	0	0	0	22	0
<i>P. rodhaini</i>	11	7	0	0	0	0	0	0	11	7
<i>P. martini</i>	19	7	1	3	0	1	0	0	20	11
Total	319	150	35	41	284	74	0	0	638	265
	46		76		358		0		903	

C. The Kibbish-Omorate focus

This focus is located at the southwestern end of Ethiopia in the lower Omo river basin. Omorate town itself is located at 400 m a.s.l. and at latitude 4°40'-4°53'N and longitude 30°5'-36°13' E. The annual average temperature is 30°C and the average annual rainfall is between 220-380 mms. The district lies between the Kitui VL focus in Kenya and the Paloich-Malakal and Setit-Humera focus in the Sudan and Ethiopia. The Omo basin covers an area of 24,600 km² which is effectively drained by the Omo and its tributaries. The total population of the district is estimated to be 26,365 (1984 census, 43). Recently, a large-scale agricultural scheme was started in an area of about 5000 hectares. The total number of employees in 1990 was 1500. There are further plans to expand the farm to even larger scales as the basin can provide an irrigable area of about 250,000 hectares.

VL surveys were carried among the Dassanetch and the Nyangatom that live around the Omo river. A total of 2,706 individuals were examined, of which 1,274 were skin tested. Serum was obtained for antibody detection. The total number of cases detected over a period of four years was three, a male child of age 2 years, a woman of age 33 years and a young man of age 29 years. The skin test and serological survey were done among the indigenous inhabitants and also among temporary and permanent labor migrants. The results are compared and presented in Tables 8 and 9. A total of 5,518 sandflies were trapped in this area in May 1991 using three methods (sticky traps, suction aspirators, light traps). *P. orientalis*, *P. martini*, *P. duboscqi* and other *Sergentomyia* were collected. *P. orientalis* was collected in larger numbers compared to *P. martini* or *P. duboscqi*. Table 10 summarizes results of female fly dissections carried during the months of May and September, 1991. At Kangatan, a village north of Kibbish where one of the three cases was living, natural infection by leishmania (yet unidentified to species level) was found in one *P. orientalis*. In a recent survey at Kangatan (Sept. 1991) another VL case has also been diagnosed.

Table 8: Leishmanin & Serological Survey Results Among Indigenous Bumes in Kibbish

Village	% Positive Leishmanin	%Positive Serology	Spleen rate
Lebere	71.0	12.0	12.6
Ataper	52.1	16.7	23.4
Natante	32.0	14.3	12.9
Naweyakori	70.0	10.0	14.9
Lukemugnén	57.6	34.4	7.6
SPCM village	55.3	11.8	9.2
All villages	56.1	17.1	12.9

Table 9: Leishmanin & Serological Survey Results in Omorate

Pop. Type	Village	% Pos. Leishmanin			% Pos. Serology		
		M	F	B	M	F	B
Indigineous	Torongole + Boarding Sch.	87.4	53.1	74.9	15	21.6	17.1
Mixed	Omorate	55.8	39.6	45.5	11.9	5.4	7.8
Farm Labourers	Camp sta. + G1	15.2	2.7	13.4	6.6	0	5.8
All Villages		42.4	3.7	40.5	9.9	9.9	9.9

Note: B = both

G1 = name of a village built for laborers

Table 10: Number of Dissected Sandflies Collected From Soil Cracks and Termite Hills

Species	Place of Collection						
	<u>Kangatan</u>		<u>Kibbish</u>		<u>Omorate</u>		<u>All places</u>
	Human bait	CDC light trap	Human bait trap	CDC light trap	Human bait	CDC light trap	Total
<u>P. orientalis</u>	*9	11	-	3	-	-	23
<u>P. duboscqi</u>	-	-	-	-	-	4	4
<u>S. clydei</u>	-	12	-	12	-	25	49
<u>S. schwetzi</u>	-	*23	1	28	-	5	57
<u>S. africanna</u>	-	1	-	1	-	-	2
<u>S. adleri</u>	-	-	-	1	-	1	2
<u>S. multidentis</u>	-	-	-	4	-	-	4
<u>S. squamipleuris</u>	-	-	-	1	-	2	3
Total	9	47	1	50	-	37	144

* Flagellates isolated by NNN medium from one fly of each species.

D. The Woitu - Kolme focus

Woitu is a small resettlement village in the Woitu river bed at 700 m a.s.l. This village was established in 1983 by the Ministry of Agriculture, and is one of the schemes categorized as "low-cost" resettlement. The Konso living on the nearby hill-top villages at Kolme have descended down to this plain. The plain is an extensive woodland and thornbush savannah traversed by the Woitu river. Long chimney type termite-hills are quite prevalent unlike in the Segen river basin where pipe-organ eroded type termite-hills prevail. The Konso living uphill had been faced with serious shortages of agricultural land and rain which has forced them to farm on the Woitu plains which can be irrigated. The resettlement was started by 134 families (347 members in 1983). Not all members

of each family moved to the plains. Although the numbers of resettlers has fluctuated each year, the overall trend is one of continual increase. The numbers fell to 84 families in 1988 when the rains were better and in 1991 it rose to 150 families (424 members).

VL survey was done in Woitu and Kolme with the view of monitoring changes in the incidence of the disease as a result of resettlement in the plains. Sera of 142 individuals from Woitu and 304 from Kolme were tested for anti-leishmanial antibodies. A total number of 1,069 individuals, 178 from Woitu and 891 from Kolme, were screened at various times (Table 11). Sandflies were collected to study the species composition. A total of 16 patients, 1 from woitu and 15 from Kolme, were diagnosed. The place of infection for the single case in Woitu was unknown. Table 14 summarizes results of the serological screening.

One hundred and ninety nine sandflies collected during June/July 1989 and Nov 1990 were all identified as species in the genus Sergentomia except for a single specimen of P. duboscqi (Table 12). No animals have yet been found to be naturally infected with L. donovani. Two strains, one isolated from Heterohyrax brucei and another from Xerus rutilus, were characterized as L. aethiopica.

Table 11: Serological Screening of VL in Woitu and Kolme, July 1989

<u>Village</u>	<u>% Positive (Serology)</u>		
	<u>Males</u>	<u>Females</u>	<u>Both Sexes</u>
Woitu	19.7	15.0	18.1
Kolme	8.5	8.8	8.7

Table 12: Sandflies Collected from Woitu and Kolme

<u>Species</u>	<u>Method of collection</u>					
	<u>Aspirator</u>		<u>CDC</u>		<u>Total</u>	
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
<u>S. bedfordi</u>	-	-	12	24	10	24
<u>S. antennata</u>	4	4	24	44	28	48
<u>S. schwetzi</u>	-	-	10	17	10	17
<u>S. clydei</u>	1	2	31	21	22	23
<u>S. africana</u>	1	1	3	3	4	4
<u>S. squamipleuris</u>	-	-	0	6	0	6
<u>S. ingrami</u>	-	1	0	1	0	
<u>S. duboscqi</u>	-	1	1	1	1	
Total	6	7	73	76	123	

E. The Gambella focus

Five field trips were made to Gambella. Four case-finding and entomological surveys were followed by one serological and leishmanin skin test survey. No autochthonous cases could be detected over a period of 7 years. Since 1983, large numbers of Sudanese refugees have come and settled in two major locations (Itang & Pugnido). The refugees all came from the eastern Sudan. About 20,000 Nuers and Dinka came from Upper Nile Province and settled at Itang. About 6,300 Ethiopian migrants, the Kembatas from Shoa, were also resettled at Baro-Abol in 1985 (Table 3). Our team decided to do the clinico-epidemiological study among these two migrant populations and also among indigeneous inhabitants (Anuaks and Nuers).

A total number of 1,398 individuals were examined of which 1,232 were skin-tested and bled for sera (Table 4). Screening for anti-leishmanial antibodies was done using the ELISA. A total number of 8 patients was diagnosed among the refugees and none among the resettlers or the indigeneous population. Another twelve patients were diagnosed among the refugees by the medical team of MSF Belgium/Holland using the Direct Agglutination Test. Large numbers of the refugees from Upper Nile province received treatment for VL. Skin-test and serology results are presented in Table 13. As is shown, the rate of seroprevalence and reactivity to leishmanin among the refugees is significantly higher than the rate among the resettlers or the indigeneous Anuaks and Nuers.

A total number of 3,333 sandflies were collected from Gambella in Itang on different trips. Results in Table 14 show the species and the methods of collection of a single field trip. All the 366 sandflies were species in the genus Sergentomyia.

Table 13: Serological & Skin-test Findings in Three Populations of Itang, 1990

Population groups	Village	% Positive (Leishmanin)			% Positive (Serology)		
		M	F	B	M	F	B
Indigenous	Itang	10.2	5.4	7.6	10.1	2.5	6.3
Resettlers	Baro Abol-A	7.3	0.0	6.1	8.5	0.0	7.1
	Baro Abol-B	1.7	0.0	<1.0	9.8	7.8	8.8
	Both	3.1	0.0	1.7	9.4	7.5	8.5
Refugees	Telule-9	35.1	3.5	34.4	16.3	15.1	15.8

M = Male; F = Female; B = Both.

Table 14: Sandflies Collected from Itang, May 1990

	Method of Collection						
	<u>Aspirator</u>		<u>Sticky traps</u>		<u>CDC</u>		<u>Total</u>
	M	F	M	F	M	F	2
<u>S. squamipleuris</u>	0	2	0	0	0	0	2
<u>S. schwetzi</u>	51	133	28	67	2	8	289
<u>S. antennata</u>	3	1	8	15	2	1	30
<u>S. clydei</u>	3	0	7	0	3	2	15
<u>S. adleri</u>	0	0	0	0	0	1	1
<u>S. africanna</u>	4	0	1	4	2	0	11
<u>S. namibensis</u>	1	0	0	0	0	0	1
<u>S. bedfordi</u>	2	12	0	2	0	0	16
<u>S. harveyi</u>	1	0	0	0	0	0	1
Total	65	48	44	88	9	12	366

Discussion

The occurrence of outbreaks of VL in Ethiopia is not well discerned especially among populations in endemic areas. In contrast, epidemic outbreaks in neighboring countries, the Sudan and Kenya, are well documented (29,45,46,47,48,49). The 136 cases reported from southwest Ethiopia by Anderson (3), patients among labor migrant in Metema-Humera region (5,6,7) and also the patients among the military in Sahil, NE Ethiopia (13) are examples of the few outbreaks known in Ethiopia. These outbreaks have resulted from migration by non-immune highlanders into the zoonotic disease foci.

Since 1980, large scale agricultural farms in northwest Ethiopia have been abandoned. Instead, new agricultural schemes have been initiated elsewhere e.g. in lower Omo basin. The lower Omo region which ecologically resembles the Metema-Humera in northwest Ethiopia and also the lowlands in eastern Sudan attracted the attention of agricultural economists and now a joint Ethio-Korean agricultural project is underway. A large number of people mainly small-scale traders and farm employees have immigrated. The indigenous inhabitants of the region (the Dassanetch) are making

a breach to their semi-nomadic pastoral life. It was, therefore, necessary to launch an active VL surveillance, including studies on the vectors and reservoir hosts of the disease.

Two nearby areas in lower Omo basin, Omorate and Kibbish, were surveyed. Our skin test surveys in those areas show a positive rate above 50% (54% in Kibbish, 75% in Omorate). Fuller et al. (9) earlier reported a rate of 61% in the same area. Positive skin test rates in school children were close to 90%. Seroprevalence was determined to be 17%, one of the highest rates from our surveys in many foci. Only three locally acquired VL cases were diagnosed despite high rates of positive leishmanin or serological reactivity. No cases have so far been identified among labor recruits of the farm. The positive leishmanin rates and seroprevalence in this group was 13% and 6% respectively. These rates, low as they are, can be expected to increase in the future. Man biting phlebotomines collected from these areas include *P. orientalis*, *P. martini* and *P. duboscqi*. The latter is known to transmit *L. major* in Kenya and Senegal (50). This parasite is believed to exist in lower Omo causing cutaneous leishmaniasis. In one recent survey north of Kibbish at a place called Kangatan, leishmania (yet unidentified to the species level) was isolated from a single *P. orientalis* (Table 10). The vectorial status of these three man biting phlebotomines in lower Omo is yet to be determined and it presents a complex ecological situation that needs to be unraveled.

Aba Roba represents a VL focus of high endemicity. Microfocal variations in the incidence of the disease are shown in Table 5. Annual incidence rates as high as 12/1000 are known in one village named Galga (Table 5b). Skin test and serological profiles (Figures 3 and 4) as well as the age and sex distribution of cases (Figures 5 and 6) suggest outdoor exposures. This is attributed to the migration of children and young adults to the Segen valley and surrounding lowlands where transmission takes place. Males are at higher risk, twice as many males being exposed or infected as females. The increasing use of the lowlands as well as down-slope resettlements in the Segen are considered to increase the extents of man-fly contact and disease.

There has not yet been any effective resettlement in the Segen valley. One planned scheme intended to resettle about 5,000 Konso has been aborted. A smaller number of Konso have been resettled in the Woitu plains. Seropositivity rates among these settlers in Woitu was the highest (18.2%) of our surveys (Table 11) although so far only one case of VL was diagnosed. The Woitu plain, even though proximate to the Segen valley, is not part of it. It is ecologically different in soil, vegetation and fauna. There is very little information about the vectors and reservoir hosts of the disease in this plain. There are cases of VL among the Konso living in Kolme and other PAs (Fig.2). These cases are considered to have acquired the infection in the lowlands of the Segen valley also as a result of temporary migrations from the highlands.

Fig.3

AGE & SEX RELATED LEISHMANIN POSITIVE RATES IN ABA ROBA

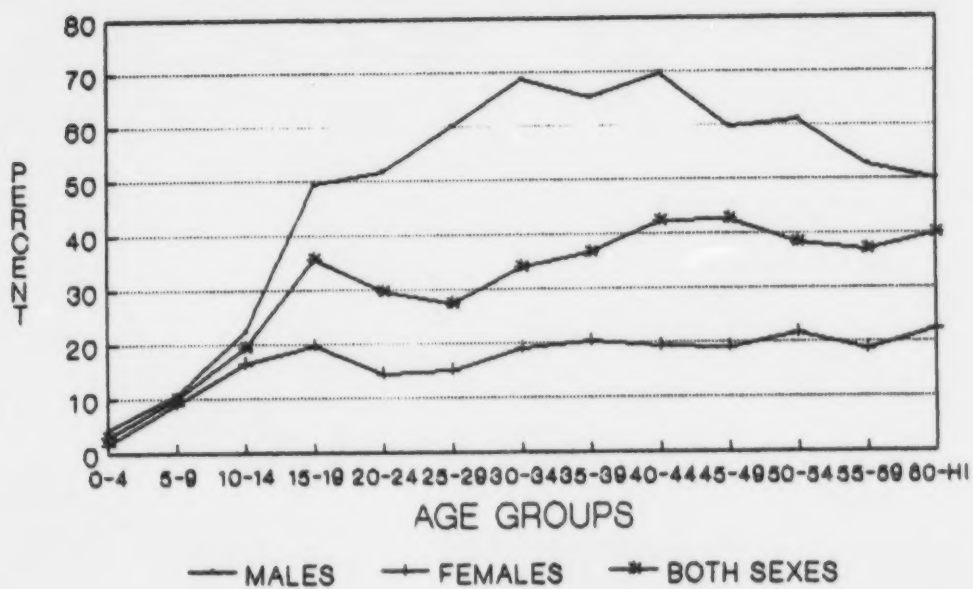


Fig.4

AGE & SEX RELATED SEROPREVALENCE RATE IN ABA ROBA

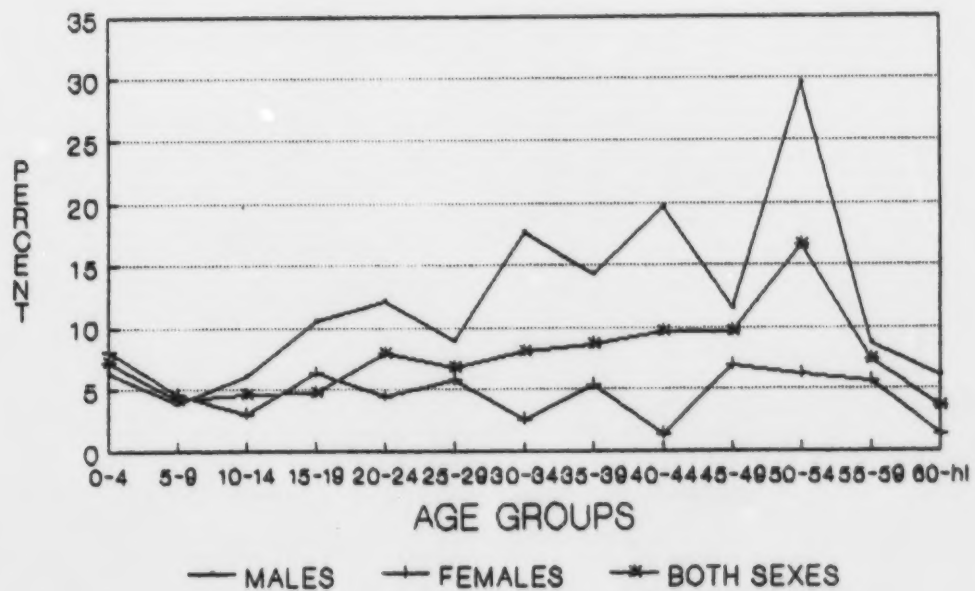


Fig.5

AGE & SEX DISTRIBUTION OF 164 CASES IN ABA ROBA

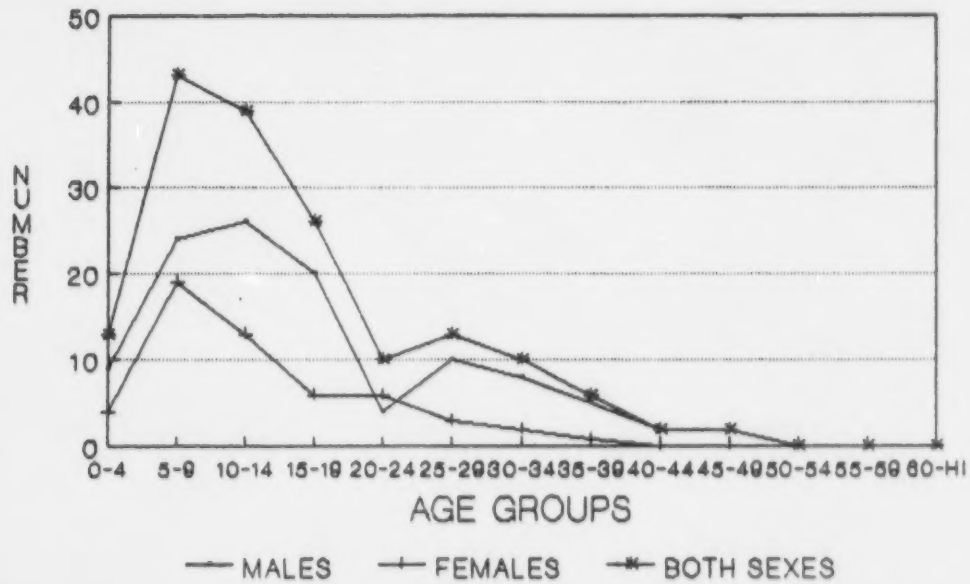
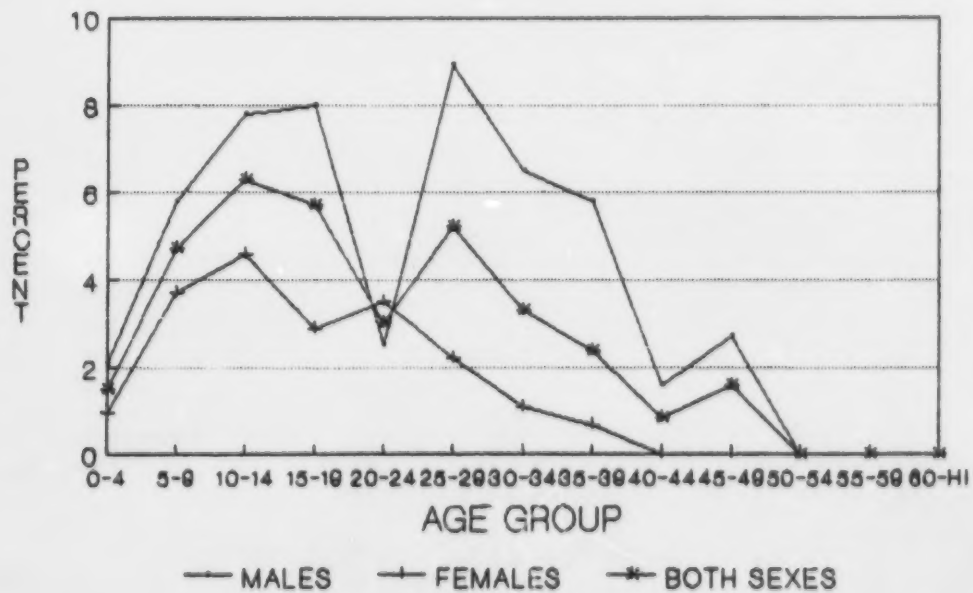


Fig.6

AGE & SEX SPECIFIC DISEASE RATES OF VL AT ABA ROBA (PREVALENCE)



A similar ecological situation exists in Gelana in South Ethiopia. *P. martini* is the suspected vector of VL in Gelana (Table 7). The disease, however, occurs only sporadically. Four adult cases of VL were diagnosed with our active surveillance programme in a community of 526 resettlers and 157 indigenous inhabitants (Table 4a). Seropositivity rates were between 3 and 5%. Positive leishmanin reactivity was also between 6% and 13% (Table 6). The seasonal abundance of *P. martini*, its biting density, and its biting habits in this focus are not known. Animal reservoirs are not known either.

Gambela is a district which, like the lower Omo region, is sparsely populated. Currently large numbers of highlanders have settled and now 55% of the population in Gambela is of settler origin (Table 3). The refugee population in 1989 was four times as large as the settler and the indigenous population. VL survey in Gambela was started in 1983 mainly in outpatient clinics at various hospitals and health centres. No cases were detected even in our latest active case-finding surveys while eight cases were diagnosed among the Sudanese refugees (Table 4) who recently originated from the Upper Nile Province in the Sudan.

Table 13 shows a summary of the serological and skin test results in three population groups, i.e. the indigenous, settler and refugees. There is a significant difference in the percent positive rates paralleling our clinical findings. No phlebotomine sandflies were found in our collections of May 1990 (Table 14). The seropositivity rates in the ELISA are as high as the rates in the Aba Roba focus. This does not, however, correspond to the leishmanin skin test or clinical findings.

Although the presence of VL in Gambela has become doubtful, our surveys have not been extensive enough to consolidate this view. It is necessary to search for potential vectors of VL, as large numbers of Sudanese refugees have had the disease before. Among Nuers and Dinka who have come from Bentiu and Pangak in Upper Nile Province, a seropositive rate of 17% and a positive leishmanin reaction of 34.4% was noted (Table 13). Perea et al. (51) also reported a seropositive rate of 18.2% using IFAT in a community in a village called Ler in the Upper Nile Province. Higher rates were also reported by De Beer et al. (49) among patients reporting to emergency hospitals in the Sudan. *P. orientalis* is implicated to be the vector of VL in the devastating epidemic areas of Upper Nile Province (Judith Schorscher, 52). One of the most important elements in the possible spread of the disease eastwards will be the increase of human populations both from the Sudan and the Ethiopian highlands. There is very little knowledge about the sandfly fauna in Gambela. Local capability in the diagnostics and in the management of VL need be improved in the hospitals and health centres of Gambella.

VL in Ethiopia is widespread in geographically varied ecological situations. There is insufficient epidemiological information in most foci. The gaps in knowledge and research needs have been identified and summarized as follows:

1. Konso

Ecology:

- Termite-hill associated
- P. martini / P. celiae are vectors
- Anthroponosis implied
- Peridomestic/extradomestic transmission
- Stable endemicity
- Large human population at risk

Research Needs:

- Transmission related vector studies
- Transmission modelling
- Surveillance and pilot control
- Search for animal reservoirs

2. Omorate/Kibbish/Kangatan

Ecology:

- Acacia-Balanites woodland associated
- Anthroponosis implied
- P. orientalis and/or P. martini are vectors
- Occurs sporadically and in outbreaks
- High seroprevalence and skin test positivity are known
- Area is sparsely populated
- Agricultural developmental schemes are expanding

Research Needs:

- Detailed studies on vector, reservoir hosts and human epidemiology
- Regional surveillance programme recommended

3. Metema-Humera

Ecology:

- Anthroponosis implied
- P. orientalis implicated
- Area is sparsely populated
- Occurs sporadically and in outbreaks
- Large-scale agriculture is being resumed

Research Needs:

- Regional surveillance recommended
- Reconnaissance surveys on vectors and reservoir hosts

4. Sahil

Ecology:

- Occurs sporadically and in outbreaks
- Area is sparsely populated
- There is very little knowledge about disease, vectors, reservoir hosts and the parasite

Research Needs:

- Active and passive surveillance
- Reconnaissance surveys on vectors and reservoir hosts
- Zymodeme analysis of parasite necessary

5. Gambella

Ecology:

- Disease is unreported or absent
- Area is sparsely populated
- Large scale settlements are underway
- Low levels of seropositivity and leishmanin reactivity suggest absence of the parasite or its sporadic nature
- Sandfly fauna is unexplored

Research Needs:

- Active case finding surveys are necessary
- Search for any potential vectors recommended

6. Woitu

Ecology:

- Area is sparsely populated
- Sporadic occurrence among recent settlers is observed
- High seroprevalence rates among recent settlers is noted
- Resettlements are in progress
- Sandfly fauna is unexplored

Research Needs:

- Annual cross-sectional surveys among resettlers recommended
- Search for vector and reservoir hosts

7. Gelana

Ecology:

- Disease occurs sporadically
- Low levels of seropositivity and leishmanin reactivity are noted
- Long standing settlements and resettlements are known
- P. martini is a suspected vector

Research Needs:

- Detailed studies on vectors and reservoir studies are necessary
- Case finding surveys on annual intervals recommended

Control and Prevention of Leishmaniasis in Ethiopia

Practical approaches towards control and prevention of VL in Ethiopia are discussed here in the context of the two epidemiological situations presented above. The lower Omo region represents an extended wide area of low incidence in the Acacia-Balanites woodlands. The Aba Roba represents a localized area of high incidence where in man-to-man transmission plays the most important role in a termite-hill ecology. As explained in the preceding pages all elements involved in the cycle of causal agents in these foci and elsewhere in other endemic areas of Ethiopia is not fully understood, making recommendations of the best strategy and tactics of control difficult. Research on control measures on a pilot-scale is necessary while at the same time conducting field epidemiological studies.

Reservoir control of VL in Ethiopia cannot be discussed as no animals are yet incriminated. In the Aba Roba focus, southwest Ethiopia, man to man transmission plays an important role for which prevention relies essentially on chemotherapy. The prospects of vector control by use of chemicals needs to be evaluated in a pilot-trial.

Anthropozoonotic or amphixenotic VL is implied in other VL endemic areas, mainly in the Acacia-Balanites woodlands. Such are areas in the southwest and northwest of Ethiopia are where the disease mostly occurs at relatively low incidence rates among the indigenous population. In such foci VL can occur in outbreaks, usually related to population movements and among labor recruits of large-scale agricultural developmental schemes. Such was the case in agricultural projects established around 1968 in the Ethio-Sudan border in northwest Ethiopia. In these foci the search for animal reservoirs has to continue and the vectorial status of *P. orientalis* or any other potential vector (e.g. *P. martini* in lower Omo) needs to be examined. Recent surveys in southwest Ethiopia have shown the presence of *P. orientalis* and *P. martini* in the same locality (IPB, unpublished data).

A VL surveillance program involving active and passive case finding surveys is recommended at a regional level within the existing health infrastructures of the Ministry of Health. A continually monitored surveillance involving peripheral health units is believed to: i) ensure the detection of changes in disease rates; ii) increase awareness; iii) facilitate easy access to drugs; and iv) to monitor the spread of the disease. Reporting by vigorous case detection and treatment of patients is necessary. Vector control by chemicals can be very difficult. A case in point was the sandfly control pilot project in the Paloich-Malakal area which proved unsuccessful; the reasons being the limitations in forest-wide application of DDT, malathion or BHK in the Acacia-Balanites woodlands.

In the Segen valley, southwest Ethiopia, where eroded termite hills provide a shelter for *P. martini*/*P. celiae* and also for small mammals, VL occurs at higher incidence rates of up to 12/1000 in localized low altitude (<1300 m) villages. This

ecological environment involves complex interactions between human agricultural activity, settlement patterns and the vector habitats. The vector itself depends on the microhabitat of other subterranean insects (the Macrotermes termites) for shelter. The feasibility of environmental control approaches e.g. destruction of termite hills, needs to be examined. Such approaches need careful consideration as their effect may in fact be in the negative direction, e.g. increasing man-fly contact. The down-slope villagization and large-scale human settlement ventures need be seriously thought out. Control of VL must be considered in the planning and implementation of any government sponsored resettlement schemes in the Segen valley. One recent resettlement scheme in the Yanda plain (a northern aspect of the Segen valley) was abandoned in 1988 when it was found out that no VL surveillance had been considered in the planning of the scheme.

The location of two villages, Galga and Goinada, at altitudes of 1200 m does not conform to the traditional Konso settlement pattern and now over 60% of VL cases in Aba Roba come from these two villages alone. About 1,500 individuals (240 households) live in these villages representing 30% of the population of Aba Roba (1990 population data, IPB). The Konso have traditionally settled on hill-top villages presumably for fear of malaria, among other reasons. The villagers on hill-tops are at lower risk of acquiring VL. Increasing population pressure and the scarcity of agricultural land has, however, gradually necessitated the increased use of the Segen valley and surrounding lowlands. The Konso have adapted to this ecological imperative mainly through temporary migrations to the plains. This down-slope migration involves night activities of variable duration: days, weeks or even months in the unsheltered expanse of the plain where termite hills are prevalent. Vector control by personal protection devices, e.g. impregnated nets, is quite feasible. Insecticide spray in the ventilation shafts of termite mounds, though difficult, is also a possible vector control approach.

As a focus of high incidence, vigorous case detection and treatment of patients in Aba Roba will remain a leading approach to control. The pentavalent antimonials (Sb^{+5}) have been the treatment of choice since the 1950s. In daily doses of 10-20 mg Sb^{+5} /kg b.wt., the drug is well tolerated even over periods of several months. Children require and tolerate higher Sb^{+5} /kg than adults. The World Health Organization (50) recommends patients with VL to be treated with pentavalent antimony in a dose of 20mg Sb^{+5} /kg b.wt. once daily to a maximum of 850mg Sb^{+5} for a minimum of 20 consecutive days. This regimen of treatment has recently been adapted for use in Ethiopian VL patients as there had not been any local drug trials to recommend a different regimen. There is need to work on this and also to search for alternative drugs.

The availability of new tools capable of detecting a few parasites in sandflies as well as in mammals, such as assays relying on DNA hybridization and the Polymerase Chain Reaction (PCR) will enable a clearer picture of the dynamics of leishmaniasis transmission in various ecological sites to be obtained. These data are required for designing effective control measures.

There is need to promote research and training towards the control of leishmaniasis in Ethiopia. There exist only a few leishmaniasis research laboratories in Ethiopia. This can handicap any efforts implementing control projects in the country and must be planned for carefully.

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The Ecology of Visceral and Cutaneous Leishmaniasis in Tunisia

M.S. Ben Rachid¹, R. Ben-Ismaïl¹, M. Ben Saïd¹

Introduction

Visceral (kala-azar) and cutaneous leishmaniasis have been known in Tunisia for many years. The first cases of cutaneous leishmaniasis were reported in the Governorate of Gafsa in 1884 by Deperet and Boinet. The world's first case of infantile mediterranean kala azar was reported in the suburbs of Tunis in 1904 by Laveran and Cathoire. Through a chronicle of the leishmaniasis (published in the records of the Institut Pasteur de Tunis) started by Charles Nicolle (1912) and regularly updated by others, we are able to follow the development of the epidemiological situation of these diseases in Tunisia from the turn of the century to the present. Beginning in the 1980s, this situation has gradually changed as a result of a severe epidemic of zoonotic cutaneous leishmaniasis in the mid-south of the country (Bouratbine-Belma 1988), where the disease had not previously been observed (Rachid et al. 1983), and the unexpected appearance of new foci of kala-azar in the same areas.

On the basis of clinical, epidemiological criteria and the occasional typing of strains, four forms of leishmaniasis can be found in Tunisia:

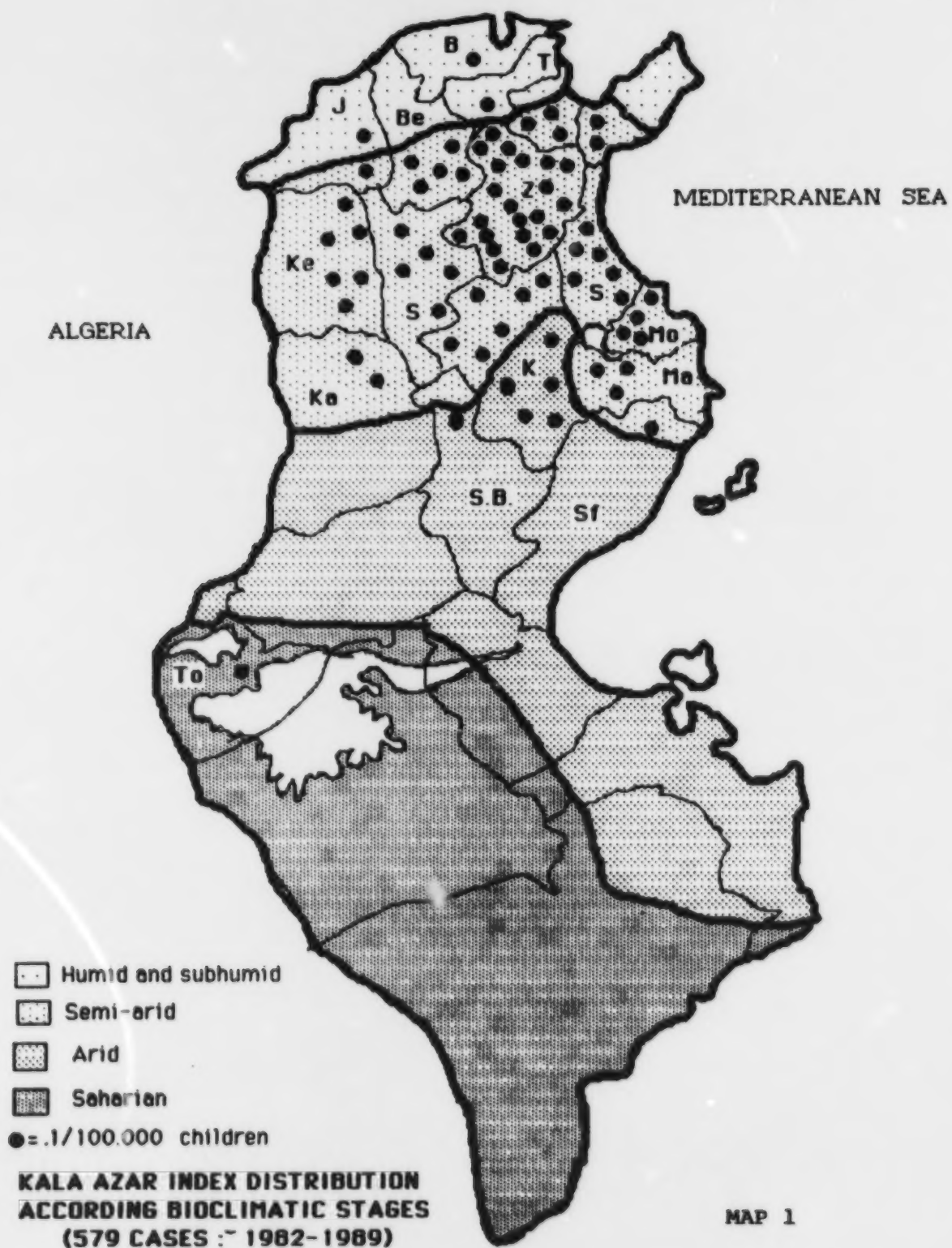
- (i) infantile kala-azar (KA) (Map 1);
- (ii) sporadic cutaneous leishmaniasis (SCL) in the North;
- (iii) zoonotic cutaneous leishmaniasis (ZCL) found in rural areas; and
- (iv) anthroponotic cutaneous leishmaniasis (ACL) in the Southeast (Map 2).

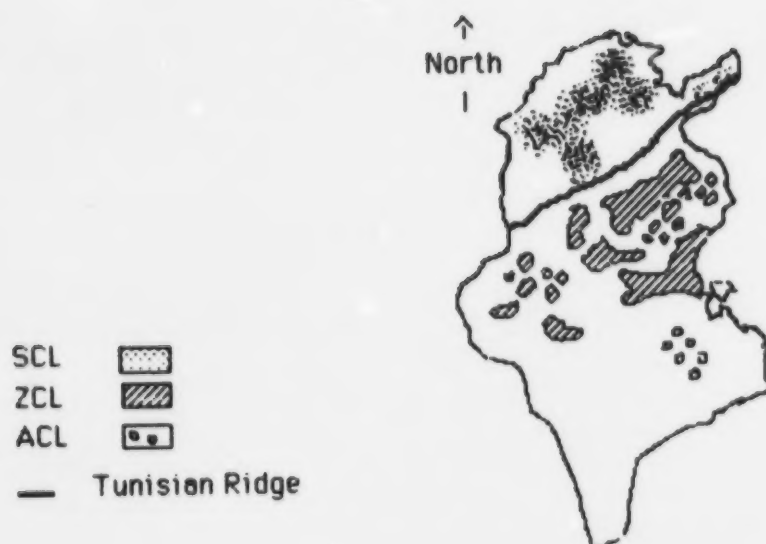
The general objective of this project² investigation was to study the role of various vectors and animal reservoirs in the transmission of these forms of human leishmaniasis in Tunisia in order to implement an appropriate strategy for the rational control of the leishmaniasis. The specific objectives were:

- (i) - to determine the prevalence of canine leishmaniasis in the foci of kala-azar and establish its relationship with human visceral leishmaniasis.
- (ii) - to confirm the theory that dogs can host strains related to cutaneous leishmaniasis.
- (iii) - to determine, in endemic strains, the role of various rodents as reservoirs of visceral and cutaneous leishmaniasis.
- (iv) - to study vector species, their density and association with the various clinical forms of leishmaniasis.

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² The Leishmaniasis Project, IDRC 3-P-86-0308.





MAP 2 : GEOGRAPHICAL DISTRIBUTION OF THE VARIOUS CUTANEOUS LEISHMANIASIS FORMS OBSERVED IN TUNISIA

- SCL : Northern sporadic cutaneous Leishmaniasis
ZCL : Zoonotic cutaneous Leishmaniasis
ACL : Anthroponotic cutaneous Leishmaniasis

Methodology

The Relationship of KA with Canine Leishmaniasis

Detection of KA (kala-azar) was passive, based on samples (bone marrow smears or serodiagnosis) that were received by us and annual statistics collected by the various pediatric health care services in Tunisia (Tunis and suburbs, Sousse, Kairouan, Sfax) and occasionally some adult services.

The relationship between canine leishmaniasis and human leishmaniasis was studied in a recent focus of KA: the Governorate of Sidi Bouzid, located in the mid-south, where six cases of kala-azar were reported between 1986 and 1988 and an epidemic of ZCL has been raging since 1982.

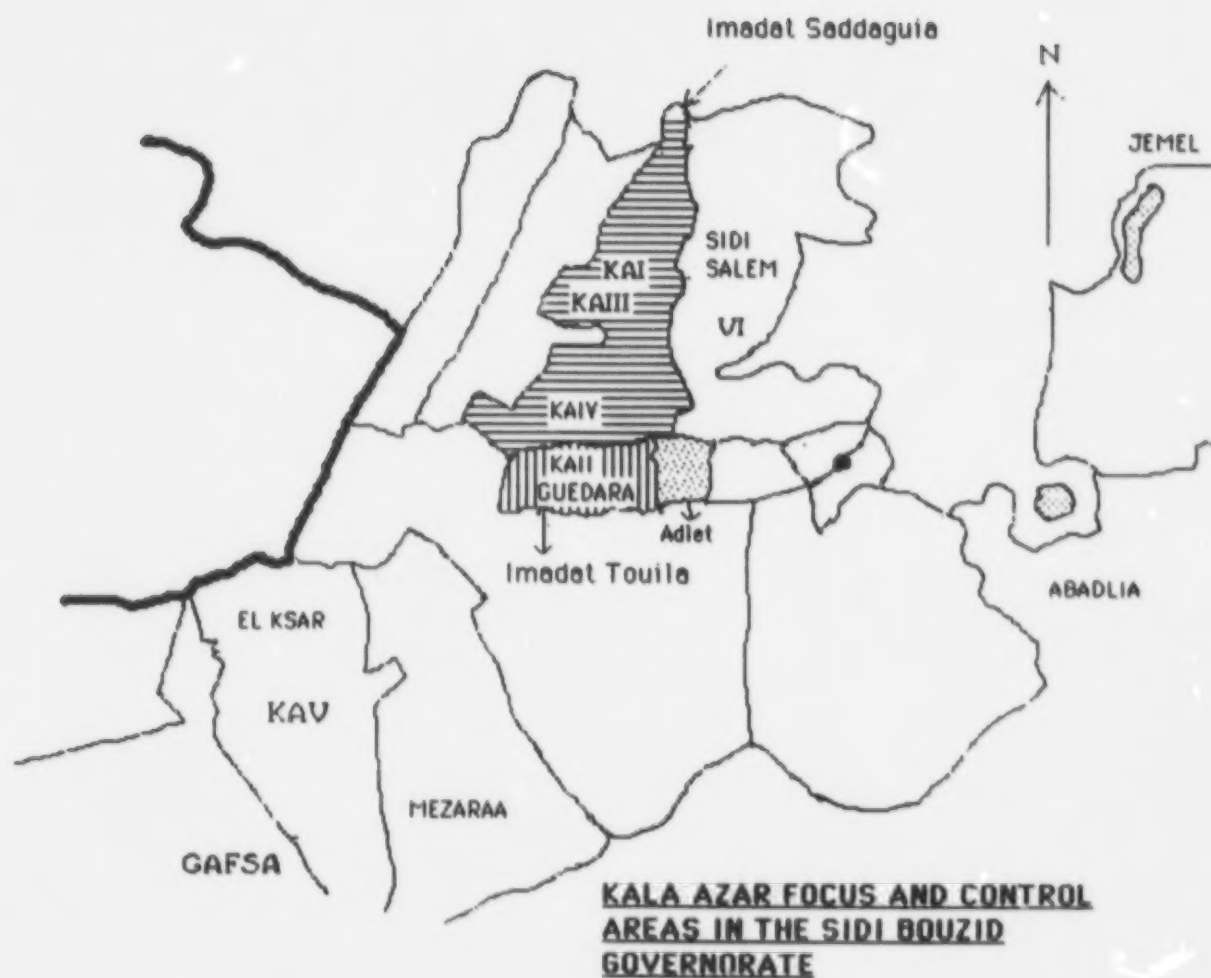
The survey of the dog population was conducted in two stages, always during the diapause seasons of the sandfly (January and April). The first stage took place in 1987 and 1988 and focused on the first four cases (I and IV), grouped in the same area; this we have called the focus area. The second was carried out in 1990, to evaluate the results of the control methods implemented during the first study and to complete the data on the other two cases (V and VI).

The first survey involved the Imadat de Saddaguia and the Imadat de Touila (Fig. 1), an area of irrigated crops covering 57 km², separated from each other by El Fekka Wadi. The total child population is 834, i.e. 14.63 children per km². Each survey took place within a 3 km radius of each patient's home (in theory, a distance equal to that of the flight of the sandfly). Three control areas without any cases of kala-azar were also investigated: Adlet on the border, Abadlia 20 km away with the same biotope as the focus and Jemel 25 km away where irrigated farming is relatively undeveloped.

After the dogs with owners had been surveyed (place, name, age, numbers), a systematic serological study was made using the IFI test (significant titer at 1/100 level, the antigen used by *L. infantum*, Tunisian strain). Samples were taken only from dogs over four months.

In 1987, we collected the data using a method that proved somewhat ineffective. In 1988, we adopted a door-to-door method with the help of (in all cases) the staff of the Basic Health Care Division (BHCD). A total of 677 dogs were tested. Positive dogs were, with their owners' consent, either bought or replaced by a healthy dog; they were then put down and autopsies were carried out to obtain smears, cultures in modified NNN medium (Brain Heart Agar Blood (BHAB)) and typing of isolated strains.

Data on agriculture and water resources programs was obtained from the appropriate divisions of the Ministry of Agriculture.



-  Control areas
-  KA focus (I to IV)

FIGURE 1

Zoonotic Cutaneous Leishmaniasis (ZCL): A Study of Rodents as Reservoirs of *L. major*

One of the main tasks of the Basic Health Care Division has been the passive detection of human cases. The role of rodents as reservoirs of *L. major* was very carefully studied in the focus of Douera located in southwest Sidi Bouzid (Governorate of Gafsa), a mixed focus of *Psammomys* and *Meriones* (Ben-Ismaïl et al. 1987).

Psammomys are captured manually during the day following the destruction of their burrows; other rodents are caught during the night using a Sherman metal trap placed near the openings of the burrows.

Specimens causing identification problems are sent in formol to the Natural History Museum (Prof. F. Petter) for accurate determination.

We looked for Leishmania in superficial cutaneous lesions, mainly on the ears but also at the visceral level: liver and spleen. Imprints were prepared and stained with MGG. A culture was made in modified NNN medium (BHAB) as well as an inoculation on the plantar cushion of a BALB/C mouse. When lesions developed on the mouse, samples were placed in a culture and the isolated strains typed.

In order to evaluate the extent to which the reservoir rodents were affected, we also tested the humoral response in *P. obesus*, *M. shawi* and *M. libicus* by using the IFI test with *L. major* as the antigen and antiglobulin homologous markers and/or a commercial anti-rat preparation.

Sporadic Cutaneous Leishmaniasis (SCL) of the North and Anthroponotic Leishmaniasis of the Southeast (ACL)

The problems caused by KA and ZCL relegated these two forms of leishmaniasis to secondary importance in the study. A survey of foci and some limited entomological studies were the main activities. We encountered, however, a specific problem in maintaining strains obtained from the primary culture of SCL lesions. We tried several methods: culture in BHAB and EMTM media, inoculating an immunodeficient golden hamster with cortisone, inoculating the BALB/c mouse infected with TG 180 sarcomatous cells (Monjour's method) and inoculating the muzzle of a three-month old dog.

We were able to obtain only one strain from a sixty-year old woman from El Haouaria (Cape Bon) who had two lesions on her lower limbs. A biopsy was performed, then crushed under sterile conditions and inoculated in Evans' "sloppy" medium. The promastigotes collected after a month were cultured in ordinary media and typed. The inoculated dog did not develop lesions during the year it was kept under observation.

The application of specific DNA probes represents undeniable progress in determining this strain.

A comparative study of the intensity of the humoral response was carried out on 173 serums from three cutaneous forms of leishmaniasis found in Tunisia. It was conducted with a dosage of IgG and IgM assays; at the same time we looked for specific antibodies using the IFI and ELISA tests with L. major, L. tropica and L. infantum as antibodies for each serum sample (Ben Saïd, Khaled, et al. 1987).

Entomological Studies

Two types of studies were conducted. The first type was designed to establish the density of the various species of sandfly. Two such studies were conducted: one in July-August 1987 in Tataouine, an area of ACL and the other in September 1988 in Sidi Thabet, located 20 km north of Tunis, a focus of KA and SCL. The method used was that of sticky paper traps: papers measuring 20 cm by 20 cm were soaked in castor oil and left at the foci before sunset and collected the following morning. The sandflies caught were put in 70% alcohol. They were then cleared in Amann lactophenol and mounted in chloral gum for identification.

The second type of study was designed to confirm the roles of the various sandflies as vectors of leishmaniasis. Two such surveys took place: one in Douara in July 1987 and the other in Nasrallah and Sidi Bouzid in July and September 1989. The sandflies caught in CDC-light traps were dissected under a magnifying glass and the *Leishmania* injected into the adipose cushions of the feet of the BALB/c mice. From these lesions (after about one and a half months), we prepared a culture in BHAB medium and conducted an isoenzymatic typing of the strains that were isolated.

Isoenzymatic Typing of Leishmania Strains

The isoenzymatic typing of leishmania strains was conducted with the cooperation of L'Instituto di Sanita de Roma (Gramiccia and Gradoni) in accordance with the procedures described by Maazoun et al. (1981).

Results

I. Kala-azar

a) **Human Statistics:** Sporadic cases of kala-azar, in the form of classical infantile mediterranean kala-azar, have occurred in Tunisia: 77% of cases were less than 5 years old, while only 2.8% of patients were over 14 years. Slightly more males were affected than females. The patients were generally from rural areas and of modest means.

The most common type of habitat is a permanent house or one made out of clumps of earth: poorly ventilated, surrounded by orchards irrigated by a nearby well and sheltered by a high cactus hedge. On the clinical level, fever, splenomegaly, hepatomegaly, wasting, pallor, pancytopenia and hypergammaglobulinemia are the most

common signs of the disease. The mortality rate still ranges from 5% to 8%, according to the health services. Table 1 shows the number of cases of KA reported in Tunisia from its discovery in 1904 until 1989. The most recent surveys (Anderson 1938; Vermeil 1956; Chadli et al. 1968; Rachid et al. 1983) emphasized the stability and grip of the foci in the sub-humid and semi-arid areas in the north of the country³ where the annual temperature is between 16-18°C and the rainfall above 250 mm; most of the cases are found in areas where the rainfall is between 600 and 400 mm per year. After a respite between 1970 and 1975 as a result of the eradication of malaria, which lead to a zero plasmodium rate in 1978, there was not only a new outbreak of kala-azar in the usual foci, e.g., Zaghouan (with an annual incidence of 20.73 cases per 100,000 children between 1982 and 1989, compared with 2.78 between 1958 and 1981) but it also spread to central Tunisia; 41.65% of total cases have occurred during the last eight years, especially in the Governorate of Kairoun, and new foci have appeared in Sidi Bouzid. The South was also affected with cases occurring in the Governorates of Sfax and Tozeur.

b) Canine Leishmaniasis: The results of the surveys carried out in Sidi Bouzid are summarized in Tables 2 and 3.

Table 2 shows the number of dogs tested in the focus during the 1987-88 survey and the number of IFI-positive dogs at a rate at least equal to 1/100. The positive rate varied between 1/100 and 1/3,200. Thirty-five dogs, out of the 380 tested in the focus, were found to be positive, i.e. 9.21%, compared with one dog in 123 in the control areas, i.e. 0.81%. The overall positive rate observed in the regions studied was 7.15%. In a recent study on canine leishmaniasis conducted using IFI and AD tests (Ben Saïd 1991) involving 265 dogs in the Enfidha region (a traditional focus of kala-azar), a plain in the North of the Governorate of Sousse on the eastern Mediterranean coast, it was noted that the figures varied from 2.94% to 16%, depending on the town. These rates are comparable to those found in our study, thereby indicating that the Sidi Bouzid region has become a highly endemic focus of kala-azar.

When we related the number of leishmanial dogs to cases of kala-azar in the focus, we found, however, that only two cases had a leishmanial dog in the home (cases II and V); within a 3 km radius of each case, however, we found between 5 and 13 leishmanial dogs per patient, i.e. about 10 affected dogs for every case of KA (Table 3).

³ Before 1956, the population affected was mainly of Italian and French origin; this observation enabled C. Nicolle to discover the reservoir of kala-azar: the dog which is found in homes of Europeans, but not in those of native people. In addition, the French and Italians were virtually the only people to develop and tend the vineyards; it is interesting to note that during the first half of the century the distribution of KA in the north of the country coincides with the wine-producing region (Map 3).

Table 1: Number of Cases of Kala-Azar (1904-1989)

Years	1904-1967 ⁴	1968-1981 ⁵	1982-1989	TOTAL
North	259	116	341	716
Center - Sahel - Kairouan - Kasserine	30	61	215	306
Mid-South - Sidi Bouzid		1	10	11
South - Sfax - Tozeur - Gabes	0 1 1	0 1 0	11 2 0	11 4 1
TOTAL				1049

Table 2: Canine Leishmaniasis in Sidi Bouzid

	1st Survey: 1987-1988		Selective killings of leishmanial dogs in 1988	2nd Survey		
	Focus: Cases I to IV Saddaghia and Guedara	Control Areas: Adlet, Abadlia, Jemel		Control of focus: Saddaghia and Guedara	Cases V and VI	Control area: Mezaraa
Number of dogs tested	380	123		101	42	31
Number of dogs IFI positive > 1/100	35	1		9	7	0
%	9.21	0.81		8.91	16.66	0

⁴ See Anderson 1938; Chadli et al. 1968; Laveran 1904; Nicolle 1912; and Vermeil 1956.

⁵ See Ben Rachid et al. 1983.



MAP 3:
GEOGRAPHICAL DISTRIBUTION
OF KALA AZAR IN TUNISIA
(1904-1956)

 Wine growing and producing area

Table 3: Number of Leishmanial Dogs Within a 3 km radius of Patients' homes: Survey 1987-88 Sidi Bouzid

Case	I	II	III	IV	Total
Number of leishmanial dogs	13	6	11	5	35
Town	Saddaghia	Guedara	Saddaghia	Saddaghia	

The second survey conducted in 1990 supplemented the data on the cases of KA not studied in 1987-88: case V in El Ksar located 15 km to the south and case VI in Sidi Salem bordering on the northeast of the focus. Out of the 42 dogs tested in these two towns, seven were positive, i.e. 16.66%. In El Mezaraa, a focus bordering on El Ksar, no dogs were found to be infected.

The control, after a year of transmission from the focus studied in 1987-88 where all the leishmanial dogs were put down, had a virtually unchanged positive rate (Table 2). At the same time four new cases of KA were noted in the region between 1989 and 1990.

c) The Isolation and Typing of Strains: All the strains isolated from patients suffering from infantile kala-azar and leishmanial dogs both inside and outside the focus proved to be identical to the reference strain OMSMHOM/TN/80/IPT1 belonging to L. infantum s. st. Nicolle zymodeme MON 1. No strain of L. major was isolated from the dogs in the Sidi Bouzid focus.

The strain MHOM/TN/87/KA, 364, isolated from a 21 month-old child suffering from KA, from Barnoussa in the Governorate of Kef, deserves special mention. Its culture in NNN was easy, unlike the strain isolated from cutaneous lesions. Its biochemical typing showed that it was a variant of L. infantum zymodeme Mon 24. This zymodeme has been isolated from Oriental Ulcer in northern Algeria (Belazzoug et al. 1985), Italy and Tunisia. This very same zymodeme was isolated in Italy in an adult suffering from AIDS-related visceral leishmaniasis.

An inventory of the sandflies found in the Sidi Bouzid region was compiled by Hellal and colleagues in 1985. Apart from the genus *Sergentomya*, four species of sandfly were present: P. papatasi (84.6%), P. perniciosus (13.2%), P. longicuspis (0.5%) and P. perfiliewi (0.04%). In order to isolate the leishmania in the sandfly, a second study was conducted in September 1989. It proved possible to isolate L. major from P. papatasi, but not L. infantum from the suspected vector, P. perniciosus.

The role of P. perniciosus as the vector of L. infantum Zym. Mon 1 has already been proven in Italy and more recently in Algeria (Izri et al. n.d.).

d) Ecology of the KA Focus in Sidi Bouzid: From preliminary studies on the ecological changes that have taken place in this region during the past few years, we were able to note the following:

- A significant increase in water resources development projects, especially surface wells. The density of wells per km² is significantly greater in the focus areas than it is throughout the governorate. As a whole, there were 0.28 wells per km² in 1976 and 0.98 wells per km² in 1987. In the focus areas, the number of wells rose from 3.53 per km² in 1976 to 13.23 per km² in 1987.

- Changes in the agricultural landscape. Irrigated farming has increased considerably since 1970. It revolves round market gardening, fodder, cereals and tree crops. The focus areas appear to specialize in market gardening and fodder crops with very few tree crops. Market gardening increased 55-fold throughout the governorate between 1974 and 1981.

We can conclude from this that irrigation and the changes in the use of farmland have compensated for the region's aridity, while helping to create a microclimate that is sufficiently humid for the survival and growth of the vector of kala-azar, Phlebotomus perniciosus, usually much more numerous in the humid areas of the North. This new focus was created when a large number of vectors and sick dogs were introduced from endemic areas at the same time.

II. Zoonotic Cutaneous Leishmaniasis (ZCL)

a) Human Statistics: Zoonotic cutaneous leishmaniasis (ZCL) seems to have always been widespread in southern Tunisia but absent in the northern and central parts of the country (Rachid et al. 1983). It results in multiple, wet cutaneous lesions on the face and limbs; they take about 3 to 6 months to heal and leave permanent scars.

An epidemic broke out in 1982 in the El Khobna Basin (Governorate of Kairouan) near the recently completed Sidi Saad dam. The disease then spread to ten governorates and nearly 30,000 cases have been recorded to date (Map 4). Epidemiological studies have enabled the identification of the parasite in humans as Leishmania major Mon 23 (Ben-Ismaïl, Gramiccia, et al 1987).

b) The Douara Focus Rodent Study: It was possible to study all the elements of the ZCL cycle in the Douara focus (Ben-Ismaïl, Ben Rachid, et al. 1987). One-hundred and twenty-six rodents were caught during this study. They belonged to the following species: Psammomys obesus (Cretzschmar, 1828): 96 specimens; Meriones shawi (Duvernoy, 1842): 14; Meriones libycus (Lichtenstein, 1910): 6; Gerbillus (G.) campestris (Le

Vaillant, 1857): 6; Gerbillus aureus (Selzer, 1956): 2; Gerbillus nanus (Blanford, 1875): 1; and Mus musculus (L.): 1.

The locations where the rodents were caught are indicated on Fig. (2). Nineteen rodents were found to be carriers with leishmanial lesions on their ears; this was confirmed by a direct examination of dermal smears. Of these 19, 15 of the 96 P. obesus (15.6%), 2 of the 14 M. shawi (14.3%) and 2 of the 6 M. libycus were carriers (Table 4).

Table 4:

SPECIES	TOTAL	INFECTED	
		No.	%
<u>Psammomys obesus</u>	96	15	15.6%
<u>Meriones shawi</u>	14	2	14.3%
<u>Meriones libycus</u>	6	2	-

A study of the antibodies in the P. obesus, found negative in the parasitological examination, enabled us to detect an extra 36% IFI-positive.

The lesions found in the Psammomys were often difficult to detect: hair loss and darkening of the skin and by palpation: infiltration and small nodules. On the other hand, those found in the Meriones were much clearer, oedematous and destructive often on both sides.

During another study in July 1989, most of the Psammomys caught had leishmanial lesions; 20/20 at Nasrallah and 8/10 in the same focus of Douara, i.e. 80% to 100% were affected.

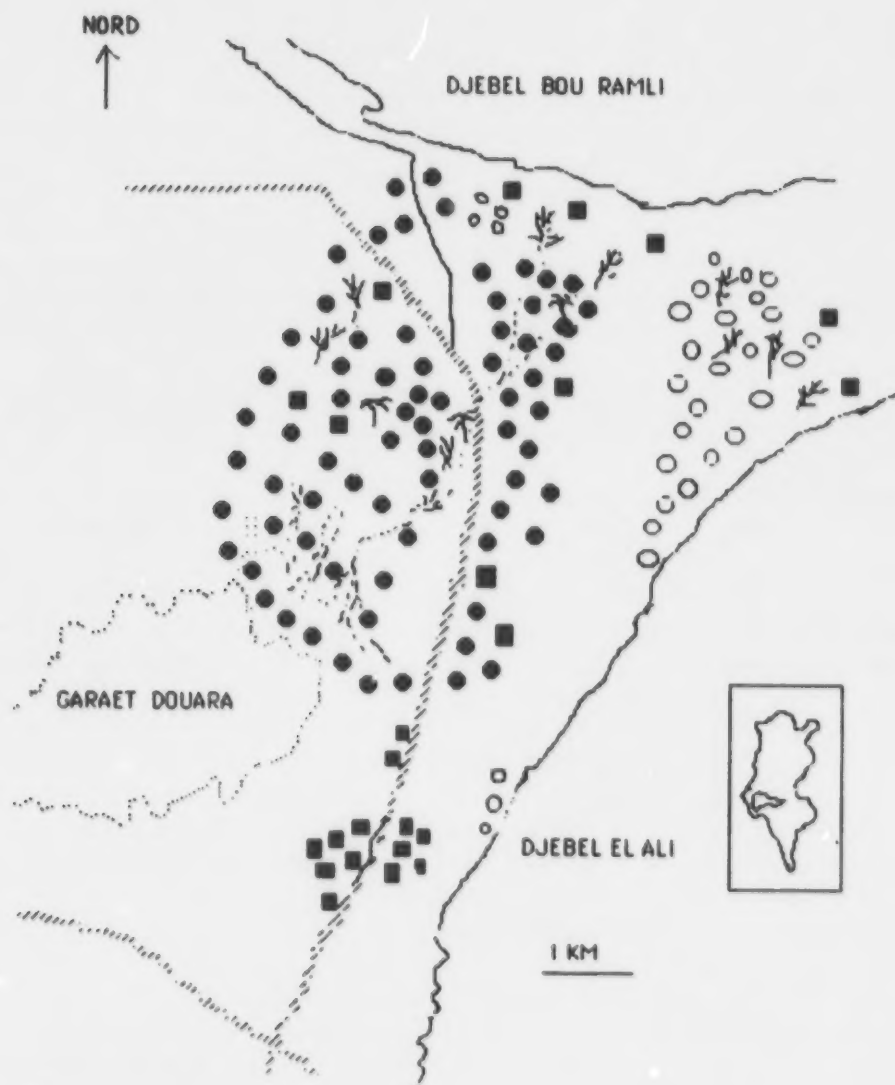
It therefore seems that P. obesus is the natural reservoir of L. major. A diurnal, prolific rodent that devours the Chenopodiaceae, it is not really a migratory animal. The greatest incidence of the disease in humans is found near its biotopes. Its distribution does not spread to the north, while in central Tunisia its distribution is exactly the same as that for ZCL. The Meriones, however, are nocturnal and migratory rodents that eat grain and are responsible for spreading the disease.

Entomological studies have identified the Phlebotomus papatasi as being the vector of ZCL (Ben-Ismaïl, Ben Rachid, et al. 1987). This species, whose anthropophilia is well known, seems to prefer a biotope of rodent burrows, where the darkness, humidity and temperature (25° C) are ideal. P. papatasi can be found in the immediate proximity of the parasite reservoir whose cycle it maintains.

All the strains isolated in this focus from the rodents P. obesus (9 strains), M. shawi (1 strain), M. libycus (1 strain), and from humans (4 strains) are identical to the reference strain MHOM/MA/81/LEM265: L. major Mon 25.



MAP 4



DOUARA ZONOTIC CUTANEOUS LEISHMANIASIS FOCUS, TUNISIA

FIGURE 2



c) The Ecology of ZCL

The construction of dams⁶ and the implementation of agricultural development programs have an impact on the agents in the ZCL cycle and transmission. According to local inhabitants, the outbreak of the epidemic in Sidi Saad was preceded by an increase in the rodent population. This can perhaps be explained by the interruption, as a result of the construction of the dam, in the flooding that occurred in the basin and routinely decimated a high proportion of these rodents every year. The enrichment of the area's ground water also helped the existing Chenopodiaceae flourish, thereby increasing the food source of the Psammomys. Another factor was the cultivation in these regions of vast quantities of Atriplex, a plant grown for sheep fodder, but also enjoyed by the Psammomys (Bernard 1971). In addition to these facts, the true rivals of the Psammomys, the camel and sheep, suffered various fates.

The camel is a multi-purpose beast used especially for ploughing and its water consumption can range from 20 to 30 litres a day; the high water content of chenopodiaceae, therefore, made an ideal food. Hence, the camel's presence provided, along with the flooding, a natural way of controlling the Psammomys. Since the camel is an encumbrance in modern farming, it is now rare in Kairouannais and central Tunisia in general. It can, however, be found in the sub-Saharan areas where it is a tourist attraction and a means of transportation in the desert.

The Tunisian sheep, itself a victim of flooding and poor reproduction, cannot seriously compete with the Psammomys. Given the lack of modern rearing methods, its very presence, in the current situation, is a factor in desertification and the atriplex crop, intended for its consumption, instead encourages the proliferation of its formidable rival.

III: Sporadic Cutaneous Leishmaniasis (SCL) of the North

This form of leishmaniasis affects the north of the country sporadically (Map 2). Its distribution pattern is the same as that for kala-azar, except that it stops at the mountain range of the Tunisian Ridge (Table 4). The annual incidence of this form can be found in Table 5.

⁶ The Sidi Saad dam was built between 1978 and 1982 to protect the Kairouan plain against the flooding of the wadis (Zeroud, Marguellil and Nebhana) that periodically devastated the region, thereby preventing any development. In 1969, for example, the huge plain was flooded with an incredible amount of water with a flow of 17,000 m³/s over a front more than 20km wide. This flooding destroyed bridges, roads, railways, power lines and homes and killed people and animals (Ministry of Equipment).

Table 5: Annual Incidence of SCL⁷

YEAR	Number of SCL Cases
1976	17
1977	13
1978	13
1979	17
1980	10
1981	7
1982	5
1983	12
1984	13
1985	31
1986	8
1987	7
1988	26
1989	15
Total	194

SCL occurs in 90% of cases as a single ulcer on the face which rarely lasts longer than 30 months. The most common lesions are small crusty ulcers, surrounded by an erythematous reaction, quite often the colour of red wine (Chaffai et al. 1988). On a parasitological level, the main characteristic of the promastigotes is that they are difficult to maintain in culture; this has for a long time prevented their biochemical characterization. The only strain that it has proved possible to type is identical in all aspects to L. infantum zymodeme Mon 24, already known in Algeria and Italy (Belazzoug et al. 1985; Ben Rachid et al. 1983). The recent application of specific DNA probes on amastigotes removed directly from lesions of eight patients made it possible to identify the causative agent of SCL as L. infantum. Italian studies indicate that P. perfiliewi is probably the vector of this form.

In the Sidi Thabet region, where both KA and SCL are found, 177 P. perniciosus were captured in September 1988, but only one female P. papatasi. The seasonal fluctuations of the various species still need to be clarified.

IV. Anthroponotic Cutaneous Leishmaniasis (ACL)

Anthroponotic cutaneous leishmaniasis (ACL) developed in the micro-foci located in the southeast between Toujène and Tataouine (Chaffai et al. 1988). Its current incidence is less than 10 cases per year. This area has not been affected by ZCL.

⁷ Statistics from the Department of Parasitology, Faculty of Medicine, Tunis.

As a rule only one ACL lesion is found (70%) on the face or limbs. The salient feature of ACL is the severity of the ulcers, which can last up to six years in some individuals. The lesions are dry and vegetated without a crust. Ulcerated forms that spread have been observed (Chaffaï et al. 1988). Unlike SCL, ACL causes high levels of serumal antibodies to form, just as ZCL does.

On the parasitological level, culture is easy. It has proved possible to type only human strains, thereby identifying the causative agent as L. tropics MON. 8 (syn.: L. Killicki) (Rioux et al. 1986). The ACL found in Tunisia is different from the L. tropica forms found throughout the world because of its non-urban distribution and the occurrence of isolated and sporadic cases, which casts doubt upon its anthroponotic nature.

In the Tatahouine region, five species of sandfly have been catalogued: (July-August 1987):

<u>P. papatasi</u>	67 specimens
<u>P. sergenti</u>	32 specimens
<u>P. alexandri</u>	17 specimens
<u>P. chabaudi</u>	9 specimens
<u>P. longicuspis</u>	3 specimens

Phlebotomus sergenti, common in the areas of transmission, is the most likely vector of ACL (Rioux et al. 1980) in Tunisia.

Control Measures

The control measures can be divided into two categories: (i) general measures and (ii) specific measures. Apart from the development of more detailed research on leishmaniasis and related subjects, general measures would include the following.

a) Training and introduction to leishmaniasis for the medical and paramedical staff of the regions concerned. Numerous courses were given in Sidi Bouzid and Mahdia that included an exchange of information about experiences and problems that had been encountered. A nationwide course took place in 1988 for the doctors and auxiliaries of the Public Health Department.

b) Incorporation of the control of the leishmaniasis as a public health problem in the operations of the Basic Health Care Division (BHCD) for KA and ZCL. By means of its outposts in the most remote areas and its close contact with the population, the BHCD is able to define its courses of action and, through health education, make people aware of the problems. With regards to KA this would require:

- monitoring and early detection of human cases in the field so that action can be taken quickly to reduce fatal effects;
- assuming responsibility for certain survey activities

- carrying out a census of the dog population so that an extensive study can be conducted;
- testing dogs; and
- making people more aware of the clinical signs of sick dogs and promoting the social acceptability of surveys and slaughtering of dogs.

Similarly because ZCL is spreading rapidly over a wide area, community participation is especially needed at various levels:

- at school, the teacher is the first to identify cutaneous lesions. By confirming the leishmanial origin of these lesions, it is possible to identify the areas affected, especially those recently surveyed.
- the village people actively participate in the manual capture of the Psammomys by pointing out the various foci of the rodents and even monitoring the equipment used in the field for capturing them.

c) **Increase government awareness** by improving communications between the Ministry of Public Health and the other ministries involved. For example, with the Ministry of Agriculture this would involve:

- developing an integrated control program against the three diseases that have ravaged the country - rabies, hydatid cyst and visceral leishmaniasis, especially active control measures against the Meriones;
- rational use of the land in risk areas by taking account of public health problems in the priorities of clearing, reforestation, and erosion prevention.
- strengthening outpost veterinary facilities so that they are comparable to those of the BHCD in human medicine.

With the Ministry of the Interior, the campaign against stray dogs must be improved by making the registration of the birth of dogs compulsory and introducing a vaccination book. With national education and information authorities appropriate action must be taken to popularize new control measures in schools and the media.

In addition to these general measures, specific control measures for leishmaniasis would include:

a) **Controlling the canine reservoir:** Selective killing of leishmanial dogs was the method we adopted in this study. Killing was carried out in 1987 and 1988 and the control of dogs in the areas affected in 1990. The findings appear in Table 2. According to these findings, there has been no change either in the incidence of KA or in that of canine leishmaniasis.

On the face of it, this measure must be considered a failure in the short term. The dog census itself might be responsible for this failure. We were, in theory, able to

survey those dogs with owners, but in approximately 40% of cases, the owners refused to respond. Carefully planned health education and legislation making it compulsory to register dogs are therefore essential; these measures will also help in controlling rabies and hydatid.

The number of stray dogs was only estimated. Since the exact number is not known, the incidence of leishmaniasis in stray dogs cannot be determined. It is therefore essential that stray dogs be put down.

Since it is impossible to conduct a large-scale survey, the selective killing method might ultimately be dangerous because it affects only those dogs with owners, in other words, those registered as having been vaccinated against rabies. Their killing might result in an imbalance in favour of dogs that have not been controlled or vaccinated.

The mass killing of dogs cannot be considered except in the case of an epidemic. Combined with treating the sick and spraying insecticides in the home, it has had some success in China. A resurgence of kala-azar occurred in that country, however, between 1968-75 following the relaxation of these measures. The ecological impact of the mass killing of dogs has yet to be evaluated.

Another available option is to treat infected dogs. Dogs are usually treated with pentamidine (Iomidine). It is reserved for dogs held in great affection by their owners; since this treatment only clears up the condition temporarily rather than curing it, it must be repeated every year. Such treatment is used in towns. It is inconceivable and unrealistic (cost, awareness) on an individual level in rural areas. In Tunisia, meglumine antimoniate (glucantime) is primarily reserved for human treatment. The use of glucantime for treating dogs in Italy has resulted in canine strains becoming resistant to antimony, thereby making the dog a permanent reservoir of leishmania.

b) Improving living conditions and providing balanced foods: Improving living conditions raises the problem of operational costs. Such improvement would enable the rational use of insecticides and the control of other possible reservoirs such as Rattus rattus, which has been found to be a carrier of leishmania in Yugoslavia, Italy and Spain.

Higher quality, better balanced diet falls within the general context of social and economic progress. KA is still a disease caused by poverty and malnutrition.

c) The value of control measures against ZCL: Control measures against ZCL result from an ecological analysis of the foci. Among the programs planned by the Tunisian government within the general context of land development or traditionally undertaken by the people, special attention is given to land that can actively contribute to controlling ZCL.

The campaign against the jujube tree, which is the traditional habitat of the *Meriones*. A project under way in Kairouannais is designed to clear the jujube trees from fertile and unused land so that crop-bearing species (almond, olive and pistachio trees) may be planted.

Another strategy has been the **reafforestation of salty and skeletal soils**. This land, which is uninhabited and not suitable for farming, remains a vast expanse where the *Chenopodiaceae* grow and the *Psammomys* actively multiply. Two types of plantation have been established there: acacia and cactus.

Acacia is regarded as a magic tree, since it is capable of forcing its roots several metres deep into the ground to draw the water and nitrogen it needs. This process, while successfully stabilizing the soil and regreening desert areas, benefits humans by providing a fodder crop for cattle. The land planted with acacia becomes shady and is no longer conducive to the growth of *chenopodiaceae*, which are a source of food for the *Psammomys* and thus for the parasite.

On the other hand, the cactus plantations that we visited had become foci for the *Meriones*. The rodents make their burrows at the foot of this spiny tree, as with the jujube, to protect themselves from predators.

The method used by the inhabitants of Sfax to till their land is also of interest in rodent control. The inhabitants of Sfax practice dry farming, which is indispensable in this country where there is so little rain, but is possible thanks to the thin soil and humid air. Olive trees are planted 20-24 metres apart. The land is worked using tractors with 7-metre wide blades which have teeth that are able to dig to a depth of 20-30 cm. Tilling is carried out six or seven times a year to reduce water evaporation, aerate the soil and destroy weeds. This tilled land has remained ZCL-free, unlike the so-called transit areas, uncultivated grazing land in the beds of the Wadi where the rodents breed.

These ecological control methods, which have already been tried and tested in some countries (e.g. the Soviet Union), make it possible to avoid using insecticides and pesticides on sandflies and rodents spread out over a wide area and therefore difficult to reach.

d) The use of pesticides: This method is particularly recommended in *Meriones* control because of the considerable damage they cause to grain crops. It is used by the Ministry of Agriculture in accordance with a procedure codified as follows: grains of wheat poisoned with zinc phosphide (1 kg of zinc phosphide, 2 litres of commercial oil for 100 kg of wheat).

Trials have been conducted with Klerat (an anticoagulant containing 0.05 grams of brodifacoum per kilogram) to get rid of the *Psammomys*. It is difficult to say whether it is effective since the results are contradictory and the operational costs considerable.

However, one has to ask the question "Is the use of insecticides justifiable?". Observations regarding *P. papatasi* (Ben-Ismaïl, Ben Rachid et al. 1987), the vector of ZCL, have clearly demonstrated that the use of insecticides in or around the home affects only a quarter of the females; the remaining three-quarters are found in rodent burrows. Chemical measures against the burrows are difficult to imagine given the extent of the area involved. The only feasible approach is to destroy the burrows.

The use of insecticides remains, however, possible against other forms of leishmaniasis, whose incidence has decreased significantly during the campaign against malaria (1968-72) (Ben Rachid et al. 1983). But in the absence of an epidemic and with only sporadic cases of KA, SCL and ACL, this use can only be on an individual basis and in the home, which is costly for people whose resources are generally limited.

e) Individual measures: Treatment is the only feasible option. Glucantime (meglumine antimoniate) is used to treat all forms of the leishmaniasis; for KA and serious forms of cutaneous leishmaniasis, treatment lasts 15-20 days involving hospitalization and for simple cutaneous leishmaniasis, "minute" treatment by intralesional infiltration. Current practice is not to treat benign forms of ZCL, which usually develop rapidly and against which people can be immunized. The use of local antiseptics will prevent additional infection. The use of mosquito nets and insect repellents is often beyond the reach of people with modest incomes.

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Tegumentary Leishmania Infection and Disease in Colombia: Evaluation of Incidence and Risk Factors

K.A. Weigle¹ and N.G. Saravia¹

Background and Theoretical Framework

American tegumentary leishmaniasis (ATL) caused by the species complex Leishmania (viannia) braziliensis is endemic throughout much of Central and South America (1). Although the manifestations are only rarely fatal, mucosal lesions cause devastating mutilations and cutaneous lesions are chronic burdens, occasionally disfiguring (2). Due to the complex life cycles of this vector-borne zoonosis control of transmission has not been possible (3). Even case management, as a means for control of the disease burden is hampered by inefficient diagnostic methods (4,5) and costly, difficult to administer, potentially toxic chemotherapy (6,7). Initial progress towards development of vaccines to prevent ATL or supplement case management has been encouraging (8-11). However our limited understanding of the protective nature of the naturally acquired immune response to L. viannia in humans hinders the development of effective vaccine strategies. (12, 13). Development, evaluation and adaptation of control programs for ATL must consider the underlying pathogenesis of this illness, which continues to be elucidated.

Observations from this project and works of others (14,15,16), including animal models, have enabled the construction of a theoretical framework for the natural history of L. braziliensis infection for residents of an endemic area, where repeated exposure to Leishmania laden sandflies may occur (Figure 1). Although not all stages of this framework are yet measurable, this framework is critical to planning future studies and control measures. Three concepts are central to this framework: 1. Exogenous reinfection is a frequent cause of recurrent lesions in endemic settings. 2. A substantial number of infections are not clinically manifest initially. 3. Latent infection is a common outcome of primary infection.

Newborns or new residents are considered to be naive hosts who have no history of contact, infection, or sensitization with Leishmania. Naive hosts, depending on their occupation, behaviors and the ecological setting of their residence may or may not be bitten by infected sandflies, represented by the transition (1) to the parasite contact stage. Dependent on host natural resistance, parasite dose, and parasite pathogenic capacity, following a parasite contact, the exposed host may either become infected or remain uninfected. Those infected either progress initially to clinically apparent lesions

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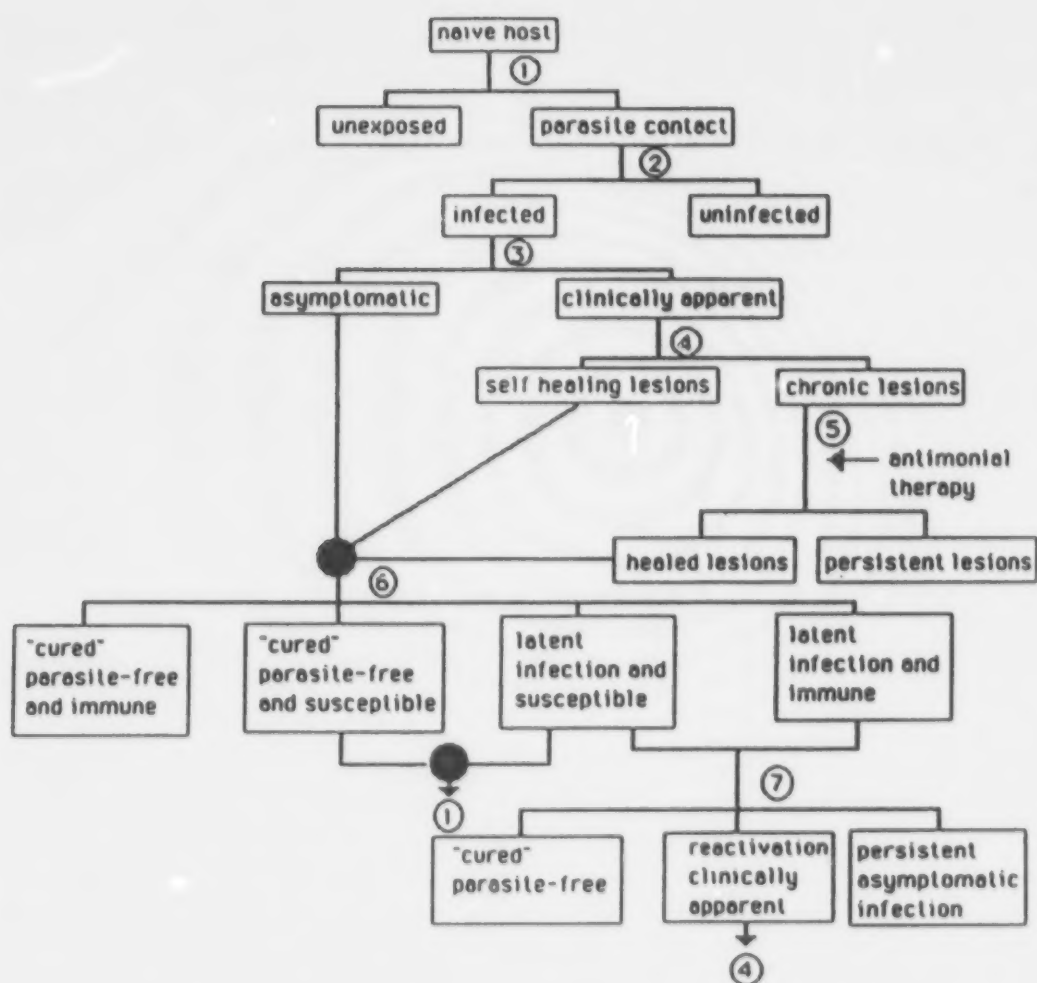


Figure 1. Theoretical Framework, Natural History of *Leishmania braziliensis* infection diagrams the most common pathways experienced by residents of an endemica area. Each box represents a potential stage in clinical progression. Transitions between stages are indicated by solid vertical lines, (stems), and are identified by encircled numbers. Nodes (●) indicate where branches may lead to similar outcomes. Arrows pointing to encircled numbers indicate that the clinical course is repeated beginning at the numbered transition.

or remain asymptomatic. Those suffering clinically apparent infections often self heal within two to eight weeks while others continue to manifest chronic lesions. The stage of chronic lesions is actually more complex than depicted. Initial lesions often heal in parallel with the presentation of satellite lesions or nearby superficial nodules, presumably infected lymph nodes. These nodules may either self heal or necrose and become verrugous nodules, or ulcers. Over time, this cycle may repeat itself. Less frequently new lesions develop distant from the primary lesions, presumably through hematogenous metastasis. Even though some lesions self heal, the host is not able to control Leishmania replication or manifestations. Furthermore, a lesion may repeatedly activate, heal and reactivate, while other lesions are continually active in the same host. Clearly there is great variety of clinical expression during the stage that is termed chronic lesions, the common feature being the continued presence of at least one active lesion. In most subjects all lesions will eventually become quiescent or healed lesions, this transition being speeded by anti-leishmanial therapy such as pentavalent antimonials. The term "healed" simply indicates that complete reepithelialization, (scarring), has occurred in all lesions and no signs of inflammation are present, the term "cured" being reserved for the absence of parasites with multiplicative potential. Rarely, persistent lesions are resistant to both host defenses and antimonial therapy and never heal.

Once all manifestations of infection are quiescent, the host is considered to have progressed to one of four possible states with respect to harboring parasites and Leishmania-specific acquired immunity (transition 6). Hosts that have completely eliminated parasites are designated as "cured" whereas hosts harboring clinically quiescent Leishmania are termed latently infected. Hosts that have acquired a Leishmania specific immune response that protects them from subsequent exogenous Leishmania reinfection are operationally defined as immune. On the other hand, hosts whose acquired immune response to Leishmanias is not protective against subsequent exogenous reinfection are defined as remaining susceptible. In reality a gradient of relative protective acquired immunity is more likely than two exclusive states. The combination of these two characteristics, parasite persistence (latency) and protective capacity of the acquired immune response, define the four possible states: Cured, immune; Cured, susceptible; Latently infected, susceptible; and Latently infected, immune. These hypothetical states will determine the potential for new lesions developing through either reinfection (re-enter cycle as "naive", transition 1) or reactivation (transition 7). Only those who have reached the cured, immune state would be expected to never experience lesions due to either reinfection or reactivation.

This framework raises numerous questions, the answers to which have direct implications for selection of control strategies for a given endemic setting. A dynamic balance between the transition states exist such that at any point in time in a given setting persons will be distributed among these possible states. For instance, in a new settlement or a zoonotic focus most persons will be experiencing transitions 1, 2 and 3. Clearly interventions aimed at decreasing infective-vector-human contact will be more cost-effective than case management. On the other hand, several years after short lived

epidemics such as that observed in Dagua, Colombia, (17) all individuals will have progressed to either chronic lesions or one of the "cured" or latent states. In such a setting neither immunization nor vector control will be as appropriate as case management. Carefully conducted longitudinal studies can estimate the rates of transition between these states and the risk factors that impact upon the probability of a particular transition. From such an understanding tools for rapid community assessments can be developed. Estimation of the incidence of clinically apparent leishmaniasis in previously naive hosts is required to predict the potential impact of primary prevention strategies such as vector control or immunization. To date the few studies that have provided incidence rates for leishmaniasis have not excluded previously infected individuals (18,19,20) or have relied on passive reporting (18). In some settings passive reporting may underestimate the incidence by many fold (19,20).

Other questions raised by this framework include: What proportion of infections are subclinical? and, What are the determinants of whether clinically apparent disease develops? In endemic areas of frequent, low level exposure to *Leishmania* strains it appears that subclinical infection is more frequent (20,21). Subclinical infection in humans by *Leishmania* of low virulence may naturally elicit protective immune responses, a phenomenon that could be replicated to advantage for vaccine development (22). The extent to which naturally acquired immune response protects against subsequent reinfection may influence the likelihood that artificially induced immunity will confer lasting protection.

Our studies and those of others have attempted to measure rates of transitions such as disease incidence rates or recurrence rates. Such parameter estimates provide an overall sense of the likelihood of such events, but may mask important sources of variations in disease manifestations. By addressing risk factors for events such as infection, clinical disease, or recurrence biological determinants for such events can be uncovered. For instance the observation that recurrences were more frequent in residents of endemic areas focused our attention upon the hypothesis of exogenous reinfection (6). Risk factors assessments can also point toward specific intervention such as modifiable risk factors, or indicate the subpopulations toward which intervention would be most efficiently directed (19,20).

The objectives of this project were as follows:

1. To determine the incidence of leishmanial infection and disease in a defined rural population residing in the Inguapi Area of Tumaco, Colombia (Figure 2).
2. Identify and measure risk factors for acquiring leishmanial infection and dermal disease in this population cohort.
3. Determine and compare the recurrence rate of tegumentary leishmaniasis in patients who have been previously diagnosed and treated in Phase I with that of individuals whose leishmanial lesions healed spontaneously, or whose primary *Leishmania* infection was subclinical.

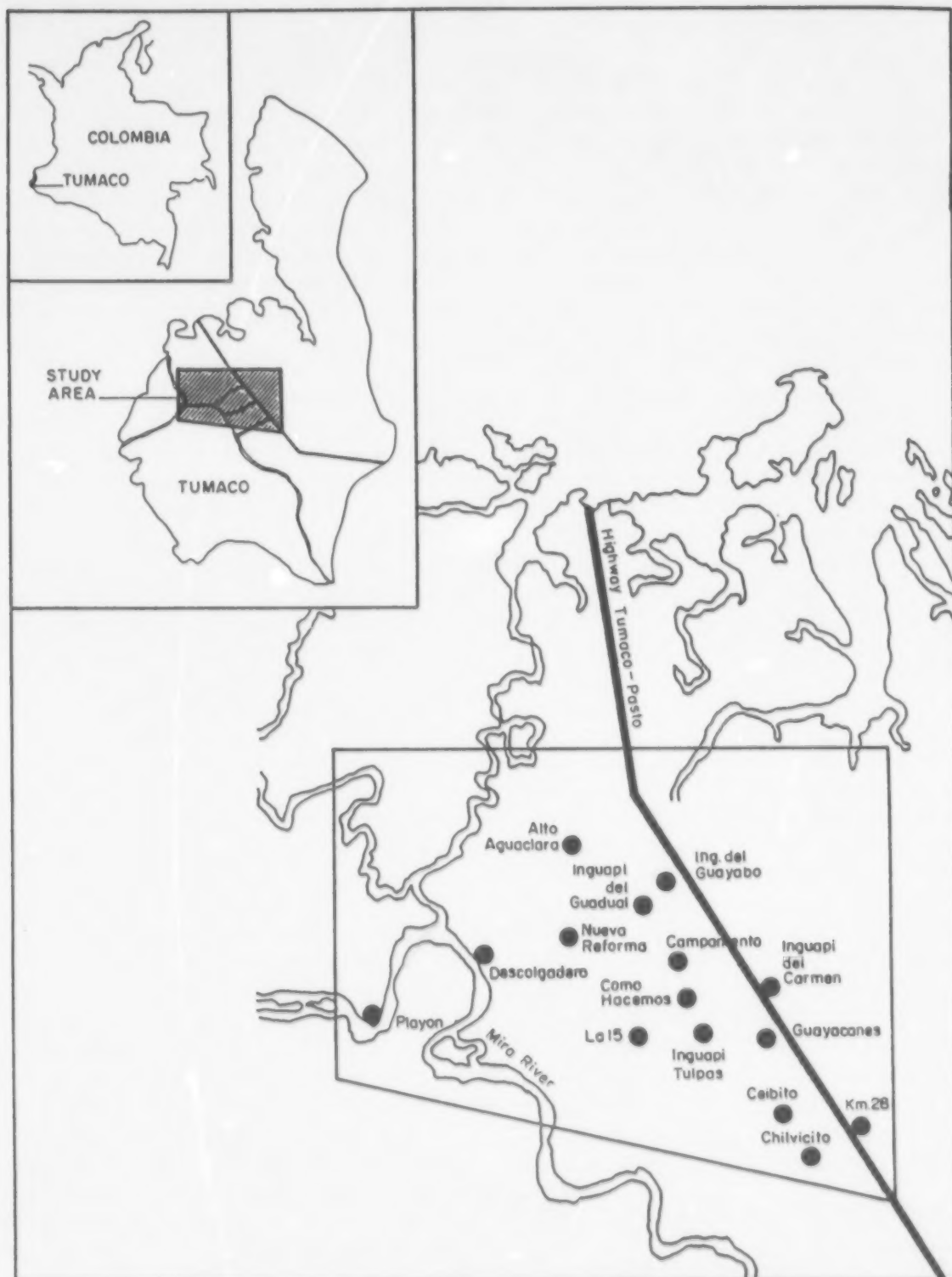


Figure 2. Insert: Location of the municipality of Tumaco within Colombia, and location of the study area within the municipality of Tumaco. Lower map: 15 villages and settlements in relation to the major road (solid black) and rivers and estuaries (double lines).

Three unanticipated issues were raised and dealt with during the course of this study. First because no standardized skin test antigen was available either nationally or internationally when this study began we were required to standardize and evaluate a skin test antigen (23). Secondly, passive surveillance for leishmaniasis proved unacceptable therefore a program of active surveillance with corresponding community involvement and an appropriate strategy for diagnostic evaluations was instituted after the first survey. Lastly a careful dissection of the molecular biology of the infecting parasite of initial and recurrent lesions revealed the diversity of pathways responsible for recurrent lesions (16).

I. Incidence and Prevalence of Leishmania bazillensis Infection and Disease

Previous studies of the incidence of ATL indicates that the rates of new cases ranges from 2.3-15.5/1000 annually in endemic settings (15,18 19). These "new case" rates are helpful indicators of the burden of disease requiring treatment in one year. However, because cases resulting from newly infected individuals were not separated from recurrences, these "new case" rates do not indicate the number of preventable cases of infection. Analogous to epidemiological studies of tuberculosis, we have utilized skin test positivity as an indicator of previous infection and skin test conversion as a marker for newly acquired leishmania infection.

The burden of leishmanial disease that is potentially preventable by control measures such as vaccines, and vector control (4), was measured as the incidence rates of newly acquired infections and lesions in a defined population residing in a Leishmania endemic area over three years. These rates are required for the evaluation and planning of such control programs. Because the mobility of the study population exceeded that anticipated, incidence rates were estimated by incidence density, which allows for varying lengths of follow-up times. However in long established foci of Leishmania transmission, much of the disease burden and source of subsequent illness may reside in previously infected individuals. Therefore the amount of prevalent infection and illness is important and was also measured in this study.

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Methods

Study Area: The study area consisted of fifteen contiguous villages settlements in the rural sector of the municipality Tumaco, Department of Nariño, Colombia (Figure 2). No cases of Chagas disease or visceral leishmaniasis have ever been diagnosed in the study area. All previous *Leishmania* isolates from the municipality of Tumaco belonged to the *L. braziliensis* species complex, being identified as either *L. b. panamensis* or *L. b. braziliensis* by isoenzyme phenotype. The study area is 27 km², between 10-30m above sea level, with 232 days of rainfall and an average of 2,600 mm rainfall annually. The mean annual temperature is 25.9°C. Ecologically the area is classified as humid tropical rain forest, although presently the villages represent a spectrum of micro-ecological conditions depending on the extent of human intervention and deforestation. The residences are constructed primarily on wooden platforms with thatch roofs, and thatch or wooden walls. The primary occupation is subsistence farming supplemented by cash crops of cacao and plantain. The cultivated fields are usually located distant from dwellings although smaller adjacent vegetable gardens are common. Agricultural workers must travel through humid forest to access their fields. The predominant anthropophilic vectors in the study area are *Lutzomyia trapidoi* and *Lu. gomezi*, infected with promastigotes at a rate of 0.1% and 0.2% respectively. Insecticide spraying is provided by the Colombian Malaria Eradication Service (SEM) and 93.9% of households in the study area reported spraying in the preceding 12 months.

Study design: A prospective cohort study was carried out, the reference population being the entire population of the 15 locales. Detailed maps of all residences within the study area were constructed, each occupied dwelling was designated by village number and household number. Acquisition of infection was assessed by leishmanin skin testing during the three surveys spaced approximately one year apart. Acquisition of leishmanial lesions was assessed during the surveys and through three years of surveillance, from the first survey until December 1, 1989.

First survey: The study procedures were explained in full. Written informed consent was obtained from all participants; parents or guardians provided consent from minors. The names, sequentially assigned numbers, age, gender, relation to head of household, and date of interview of each household were recorded. For each resident an Individual Questionnaire regarding socio-demographic behavioral and occupational risk factors was completed. A complete exam of the skin and the upper respiratory

mucosa was performed and a leishmanin skin test applied, and read 48-54 hours later (23). In addition, the head of the household, defined as the individual providing the greatest economic support within the household, was queried as to the size, location, and ecological conditions of the family's landholdings, use of insecticide, and recognition of vectors. The construction and ecological environment of the residence was recorded after direct observation.

Second survey: Approximately one year later, changes in family composition were noted. In addition, a Transfer Form was completed for each individual whose location had changed since the first survey, noting the dates of departures arrivals, birth, and the village and household of both prior and new place of residence. All participants were examined for leishmanial lesions and LST reactivity was determined for all prior residents who had not reacted to the LST during the first survey, and for new residents.

Third survey: One year after the second survey, transfers were again noted, and physical exams repeated for both new residents and residents remaining from the prior surveys. All present residents were skin tested, regardless of their reactivity to prior skin tests.

Leishmaniasis surveillance: Upon completion of the first survey, the head of household was provided an inscription card listing all surveyed household members and briefly explaining the leishmaniasis surveillance, diagnostic, and treatment program. Families were enrolled in a leishmaniasis surveillance program consisting of heightened community awareness and recognition of leishmanial lesions. In each locale, one or two health promoters were taught to recognize, record, and refer such cases to the survey team. In addition, a member of the survey team visited each village twice monthly to review the case finding records with the promoter and evaluate persons with skin lesions.

Physical exams: Participants were provided either gowns or loose shorts after disrobing. Scarred lesions and active lesions were recorded as described in Table 1. The initial classification of active lesions served to guide diagnostic evaluations described in the following section.

Diagnosis of leishmaniasis: Due to the intensive surveillance and early evaluation of lesions, we intentionally evaluated many more lesions than were likely to be caused by *Leishmania*. In an attempt to diagnose all lesions caused by *Leishmania*, we evaluated lesions ranging from characteristic to atypical. Many of the latter were self-healing and/or bacterial in origin. Individuals who were LST reactive, presented with typical lesions, or presented with non-purulent lesions were evaluated diagnostically. Individuals with purulent lesions were treated with systemic antibiotics and local debridement. Diagnostic evaluations were performed for suspect or atypical lesions that did not heal by two of four weeks, respectively. Diagnostic evaluation consisted of dermal scraping, histopathology and culture of aspirate with the addition of the inoculation of biopsy material into hamster in selected circumstances as previously described (4). The LST was applied at the time of diagnosis. Using this protocol, it is possible that suspect or atypical, spontaneously healing lesions went undetected, but unlikely that clinically important lesions were missed.

Table 1: Criteria for Active and Healed Skin Lesions
Initial Clinical Classification

<u>Active lesions</u> <u>criteria</u>		<u>Healed lesions</u> <u>criteria</u>	
1. No history of trauma 2. During over 2 weeks 3. Round or ovale ulcer 4. Ulcer with raised borders 5. Smooth nodule 6. Satellite lesions 6. Scar hypo or hyper pigmented 7. Localized adenopathy		1. No history of trauma 2. Duration over 2 weeks 3. Round or ovale scar 4. Smooth surfaced scar 5. Depressed scar	
<u>Number of</u> <u>Criteria</u>	<u>Classification</u>	<u>Number of</u> <u>Criteria</u>	<u>Classification</u>
>5	Typical	>5	Typical
3 or 4	Suspect	3 or 4	Suspect
<3	Atypical	<3	Atypical

Diagnostic categories, active lesions

- P = Parasitological, Leishmania isolated or visualized
- C = Clinical - lab supported meeting for of the following
1. Clinical classification either typical or suspect
 2. Histopathology compatible with leishmaniasis; granuloma or mononuclear, histiocytic chronic inflammatory infiltrate
 3. LST reactive
 4. Other diagnoses excluded (Sporotrichosis, Cancer, Leprosy)
 5. Response to antimonial therapy
- IM = Immunologically supported - meet two criteria
1. Clinical category either typical or suspect
 2. LST converted at time of diagnoses
- L = Likely diagnosis, does criteria for P,C or IM diagnostic categories, clinical classification is typical or suspect and meets at least one of the diagnostic criteria #2-5 of the clinical-lab diagnostic category.

The definitions of the four diagnostic categories employed are displayed in Table 1. Although all four of these diagnostic categories are at least as strict as those currently utilized in epidemiological studies of leishmaniasis (15, 21), they were devised to represent increasing levels of certainty regarding a leishmanial etiology from likely, immunological, clinical-lab to parasitological categories. Cases which fit any of the four categories were considered as diagnosed leishmaniasis.

Outcome definitions: A prevalent case of leishmaniasis was defined as the presence of active lesions of diagnosed leishmaniasis at the time of the first survey in which an individual participated. A prevalent infection was defined as LST reactivity at the time of a participant's first survey. An incident infection was defined as skin test conversion, from LST non-reactive to LST reactive. An incident case of leishmaniasis was defined as lacking active skin lesions during their first survey who later developed active lesions diagnosed as leishmaniasis. Incident cases were further subdivided based on prior LST reactivity clinical history and prior skin lesions. An "Incident lesion and Infection" (I.I.) was defined as previously LST non-reactive, lacking a scar during prior surveys and lacking a typical scar and clinical history of leishmaniasis. An "Incident lesion, but Pre-existing infection" (I.P.) was defined as being LST reactive prior to lesion onset but lacking a prior characteristic scar or history of leishmaniasis. When new lesions presented in someone with a prior history or scar typical of leishmaniasis who was known to be LST reactive prior to onset of the new lesions, the case was defined as "Recurrent lesion, Pre-existing infection" (R.P.). New skin lesions presenting in individuals with pre-existing typical scars and/or history, who were skin test negative prior to the onset of the new lesions were defined as "recurrent lesion, new infection" (R.N.). These four definitions were operational and do not necessarily imply a particular sequence of pathogenesis.

Person time calculations: Time at risk was calculated differently for infection rates. Persons who remained LST non-reactive were considered to be at risk for infection during the time interval between two or three non-reactive skin tests. New residents were considered to be at risk for infection after their first negative LST with the exception of newborns who were assumed to be at risk from day of birth. Persons who moved from one locale to another were considered to have contributed person time to their first locale until the date of the move. Persons whose skin test converted were assumed to be at risk for infection from the date of their first negative LST until the date of skin test conversion, which was estimated as the midpoint between the negative and positive skin test.

With the exception of newborns, individuals were considered to be at risk for leishmaniasis from the date of their first physical exam until the date of the first censoring event. Censoring events included: 1. Onset of new skin lesions diagnosed as leishmaniasis; 2. Departure from study area; 3. Refusal or absent for yearly physical exam; 4. End of surveillance program on 12/01/89. Infants born during the study who were enrolled and examined were considered to be at risk from day of birth until the first censoring event. When departure date was unknown it was estimated to be 6 months after the preceding survey in which the individual participated.

Results

Study population: In total 2858, 2714, and 2252 individuals participated in the first, second, and third surveys respectively. Between the first and second survey 742 residents left the area. During the second survey 598 new participants, including 110 newborns were enrolled. Between the second and third surveys 589 residents left the area. During the third survey 127 new participants, 104 of whom were newborns were enrolled. By the third survey 1771 (62%) of the original participants remained in the study area.

Nearly all residents listed on family registers participated in the skin exam and LST (Table 2) only 202 declined participation. Most of the non-participants were absent during time of visits to household. Villages with lower participation were located along waterways and highways and had a greater degree of absenteeism. Participation rates were greater for children and women who are less frequently absent from the home. Because the LST requires two appropriately timed contacts, data on LST results are available for fewer persons than were interviews and physical exams. Of those interviewed in the first survey, 98.7% (2822/2888) also underwent the physical exam; whereas LST results are available for 92.9% (2656/2888). The rates of participation were nearly identical for the three surveys.

Cumulative Prevalence of Infection: Villages varied considerably with respect to the percent of residents previously infected, based on the prevalence of LST reactivity during the first survey (Table 2). The villages with lowest prevalence are located along highway and river borders where the dense population has virtually eliminated forest vegetation. The highest prevalence was observed in dispersed settlements, Campamento, and La Quince, in which most households are surrounded by tropical forest. The rank of villages with respect to prevalence remained constant during the three surveys. Cumulative prevalence of infection increased with age, reaching a plateau at 30 years of age. Prior infection was three times greater in males than females.

Incidence of infection: The incidence rate of infection was 6.6/100 person-years at risk overall and greater in the first year. In general, villages with a greater prevalence also were found to have higher incidence rates ($p < 0.1$). One exception to this correlation is the village La Quince which was recently settled, thus explaining the lack of cumulative prevalence of infection but the high acquisition at infection. The greatest rates of infection were in those between 30 and 60 years of age. Incidence was greater for males than females, $RR = 1.7$ (8.5/4.9).

Leishmaniasis cases: More than half of the diagnosed cases of leishmaniasis demonstrated in active prior lesions at time of enrollment (Table 3). Of the prevalent cases, three were actually recurrences of cases diagnosed during Phase I. *L.b. braziliensis* was isolated in only two prevalent case, whereas all remaining isolates from both prevalent and incident case were characterized as *L.b. panamensis*. The majority of

Table 2: Participation and prevalence of leishmanin skin test (LST) reactivity during the first survey by village.

Village	Population	Interviewed		Skin tested		LST reactive	
	n	n	% ¹	n	% ²	n	% ³
Inguapí del Guadual	370	357	96.5	346	96.9	40	11.6
Inguapí del Guayabo	86	86	100.0	82	95.3	7	8.5
Nueva Reforma	255	242	94.5	228	94.2	41	18.0
Campamento	33	33	100.0	33	100.0	5	15.2
Como Hacemos	14	14	100.0	10	71.4	2	20.0
La Quince	58	58	100.0	57	98.3	2	3.5
Inguapí las Tulpas	132	132	100.0	127	96.2	15	11.8
Alto Aguaclara	217	186	85.7	134	72.0	18	13.4
Descolgadero	330	286	86.7	272	95.1	4	1.5
Playón	307	225	73.3	198	88.0	1	0.5
Guayacanes	320	295	92.2	254	86.1	16	6.3
Ceibito	200	200	100.0	193	96.5	28	14.5
Km 28	335	286	85.4	273	95.5	28	10.3
Chilvicito	92	89	96.7	88	98.9	8	9.1
Inguapí del Carmen	405	369	91.1	361	97.8	31	8.6
Total	3,154	2,858	90.6	2,656	92.9	246	9.3

1 = % of population interviewed

2 = % of those interviewed whose LST was applied and read

3 = $\chi^2 = 76.9$, d.f. = 12, $P < 0.0001$

Table 3: DESCRIPTION OF DIAGNOSED CASES OF TEGUMENTARY LEISHMANIASIS

	Prevalent Cases	Incident Cases
Diagnostic Category	-	
Parasitologic	35	19
Clinical - lab	5	2
Immunologic	0	9
Typical	3	4
TOTAL	43	34
Parasite identification		
L. b. braziliensis	2	0
L. b. panamensis	23	14
TOTAL	25	14
Classification incident cases		
Incident lesion and infection (II)		24
Incident lesion, pre-existing infection (IP)		1
Recurrent lesion, pre-existing infection (RP)		6
Recurrent lesion, new infection (RN)		3
TOTAL		34

incident cases lacked prior lesions and were LST non-reactive at enrollment. Four of the six cases who demonstrated both prior healed lesions and LST reactivity at enrollment were actually recurrences of cases originally diagnosed during Phase I.

Distribution of cases over time: During the diagnostic evaluation of individuals presenting with new lesions, the duration of the presenting lesion was obtained. Based on the date of the diagnostic evaluation and lesion duration, the date of onset was estimated. Seasonality was not apparent in the pattern of lesion onset (Figure 3). Many of the non-diagnosed cases appeared during the largest cluster of cases 5/88-11/88 indicating that our diagnostic categories probably excluded several cases of leishmaniasis.

Incidence of leishmaniasis: Overall the incidence of diagnosed cases or leishmanias who presented with new lesions either during surveillance or yearly surveys was 4.6 cases per 1000 person years at risk, Table 4. There was a large variation by locale in the incidence rate, however, the highest overall incidence rate observed was 20 per 1000 person years. Hence, the study area should be considered to be of low to moderate endemicity. The incidence of illness was correlated with the rate of infection of each village. (correlation coef.=0.69, $p=0.004$) this would indicate a relatively constant rate of pathogenecity across villages. Overall, for every 100 newly infected individuals 9.25 were diagnosed as having leishmaniasis during the first two study years. The incidence rate of leishmaniasis was relatively constant year to year. The highest incidence of leishmaniasis was observed in the ten to twenty-nine years old age group and in males (Table 5).

Relationship between pre-existing LST reactivity, and scars to risk of leishmaniasis: Risk of illness was greatest in persons who were previously infected (LST reactive) and had a typical scar at time of enrollment (Table 6). Prior to enrollment these 213 individuals had evidently suffered a clinically apparent infection. Then during the three years of surveillance six of these individuals developed a recurrence. Even for initially LST non-reactive individuals, the presence of a typical scar was associated with a three fold increase in risk. However, for persons lacking a typical scar, pre-existing LST reactivity did not confer an increased risk of leishmaniasis. These individuals had already demonstrated their ability to confront Leishmania infection without manifesting skin lesions and demonstrated an illness rate similar to that of persons without clinical immunological evidence of prior contact with Leishmania. The largest fraction of new cases of leishmaniasis developed in previously naive hosts (attributable risks = 70.6%)

Discussion

In the study area Leishmania infection was much more common than disease both at enrollment and during the longitudinal follow-up period. It is uncertain as yet whether the low pathogenecity observed was due to parasite characteristics, parasite dose or host characteristics. In other settings we and others have observed a larger fraction of the clinical manifestations in LST reactive individual. However, most prior studies were

Figure 3. Frequency of leishmaniasis by time of onset of lesions and diagnostic criteria.

I. Only parasitologically diagnosed cases

II. All diagnosed cases (categories, P, C, IM and L)

III. All evaluated cases

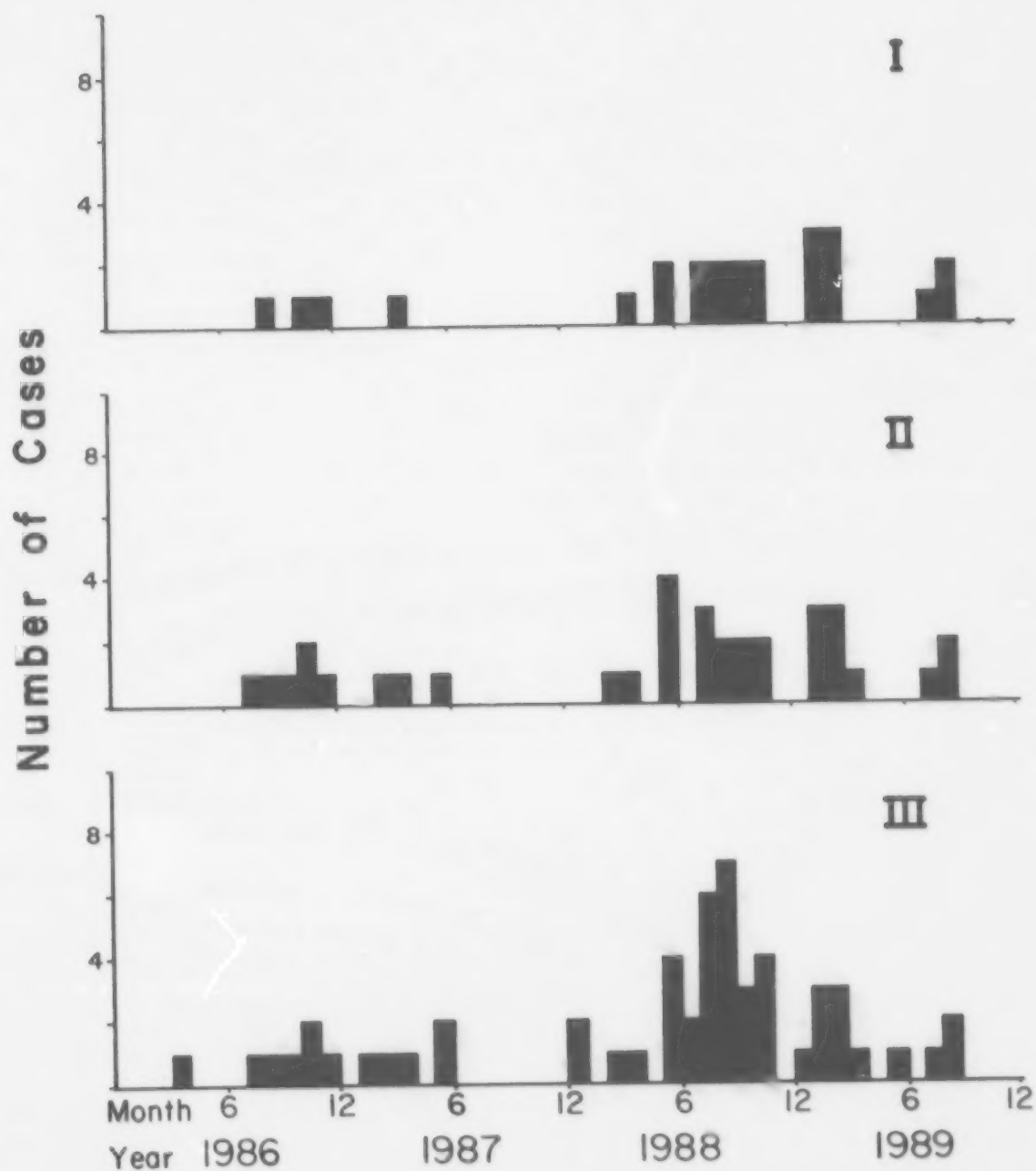


Table 4: INCIDENCE RATE OF TEGUMENTARY LEISHMANIASIS
BY VILLAGE OF RESIDENCE

	Year of Observation			Total		
	1st	2nd	3rd	-	Cases	Person
	Rate*	Rate	Rate	Rate	(N)**	Years
Inguapi del Guadual	15.2	3.2	6.1	8.3	(8)	965
Inguapi del Guayabo	0.0	0.0	15.6	5.0	(1)	200
Nueva Reforma	9.2	21.6	16.1	15.7	(11)	699
Campamento	0.0	38.5	0.0	12.3	(1)	81
Como Hacemos	0.0	0.0	0.0	0.0	(0)	9
La Quince	0.0	37.0	34.5	20.4	(2)	98
Inguapi las Tulpas	0.0	0.0	10.2	3.1	(1)	320
Alto Aguaclara	0.0	0.0	7.0	2.2	(1)	456
Descolgadero	3.7	8.2	4.1	5.3	(4)	757
Playon	5.3	0.0	0.0	1.7	(1)	600
Guayacanes	0.0	0.0	0.0	0.0	(0)	707
Ceibito	0.0	9.6	0.0	3.5	(2)	571
Km 28	0.0	0.0	5.2	1.5	(1)	662
Chilvicio	0.0	0.0	0.0	0.0	(0)	226
Inguapi del Carmen	0.0	0.0	5.0	1.1	(1)	892
TOTAL	3.6	4.8	5.8	4.7	(34)	7,243

*Rate per 100 persons-years at risk

** = Number of incident case of leishmaniasis

Table 5: INCIDENCE RATE OF TEGUMENTARY LEISHMANIASIS
BY AGE AND GENDER

Age Group (Years)	Year of observation			Total		
	1ST	2ND	3RD	Cases	Person	
	Rate*	Rate	Rate	Rate	(N)**	Years
0 - 9	0.0	1.1	3.3	1.4	(4)	2782
10 - 29	5.9	9.8	10.7	8.6	(20)	2320
> 30	5.3	4.2	4.5	4.7	(10)	2141
TOTAL	3.6	4.8	5.8	4.7	(34)	7243
Gender:						
Female	0.8	4.1	2.7	2.5	(9)	3603
Male	6.2	5.6	9.0	6.9	(25)	3640

*Rate per 1000 person years at risk

** = Number of incident cases of leishmaniasis

Table 6: INCIDENCE OF LEISHMANIASIS BY INITIAL IMMUNE AND CLINICAL STATUS

Status	Cases	Person Years	Rate*	Relative Risk	Attributable Risk
Scar Present**					
LST Positive	6 (RP)***	213	28.2	7.13	17.6%
LST Negative	3 (RN)	240	12.5	3.16	8.8%
Scar Absent:					
LST Positive	1 (IP)	394	2.5	0.642	2.9%
LST Negative	24 (II)	6074	3.9	1.00	70.6%

* = Rate per 1000 person years at risk

** = Typical scar present

*** = Case category

cross-sectional and thus unable to consider the dynamic and varied natural history of tegumentary leishmaniasis (19,21). In this study area a considerable fraction of incident cases of leishmaniasis (as many as 26%) may not be preventable by measures that attempt to reduce infections, because these individuals are already infected. Furthermore, the number of prevalent active cases exceeded the number of incident cases observed over three years, illustrating that for this chronic, recurrent infection, control programs must address both the infections and the cases that have accumulated over the preceding decades. In more highly endemic settings where up to 80% of the population are already infected, disease reduction programs may need to examine chemotherapy or immunotherapy to control manifestations in previously infected persons.

Given the low overall incidence rates observed in this study (6.6 and 0.47 per 100 person-years respectively for infection and disease) community-based primary prevention programs may not be warranted. Rather, secondary prevention programs should control the progression of clinically manifest lesions and primary prevention should be targeted toward individuals at risk.

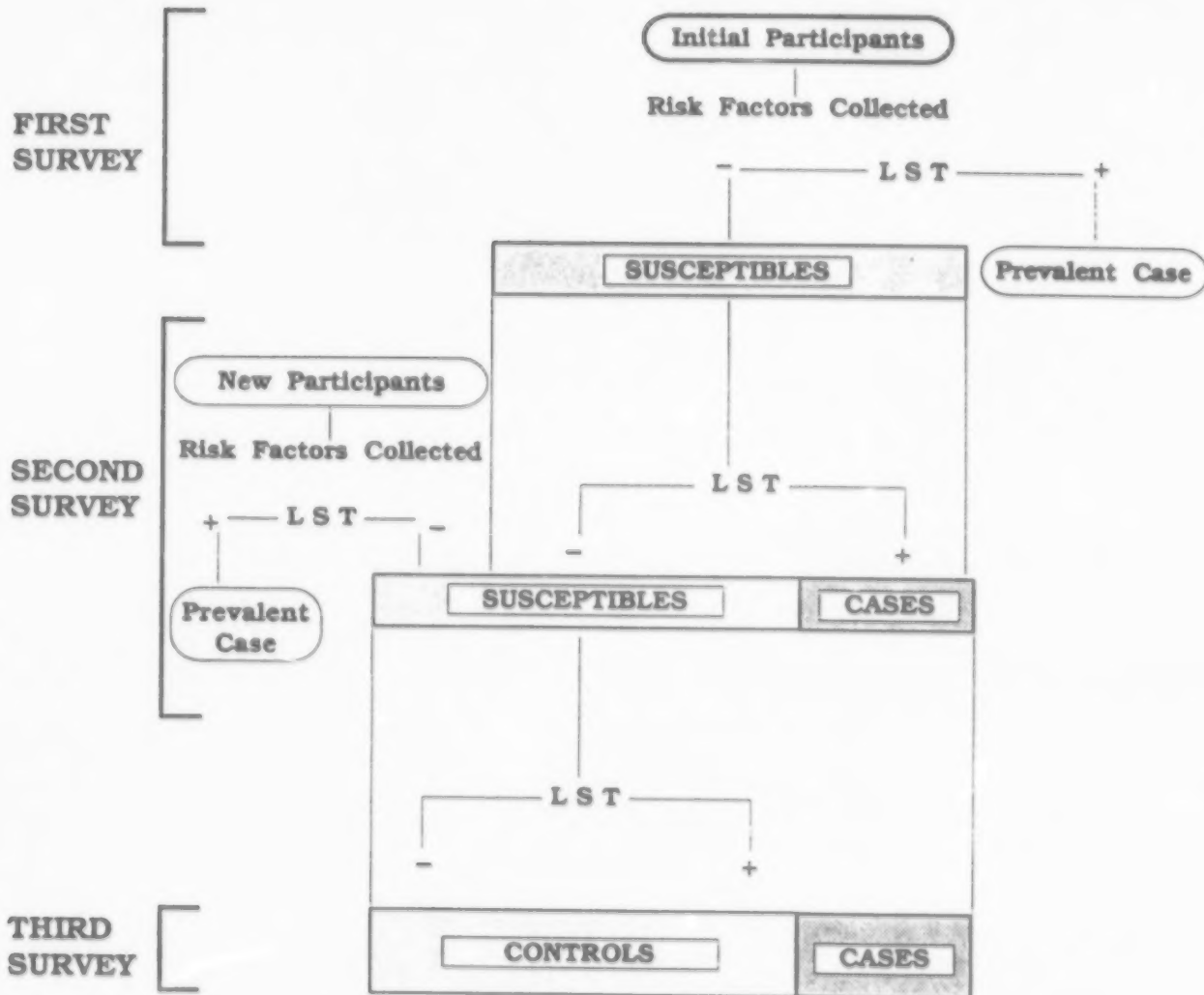
II. Risk Factors for leishmanis Infection and Disease

Cross sectional studies and case series have indicated that populations with frequent contact with the habitat of infected sandflies are at an increase risk of ATL. Such studies have focused primarily upon adult men and occupational groups. After a review of existing literature and a pilot-study we sought to examine risk factors that might be associated with intradomestic, peridomestic, and occupational transmission. Based on our pilot study and reported cases we believed that transmission in the study area was primarily peri-domestic and extra-domestic. However, we also examined risk factors related to intra-domestic transmission. Conduct of a population based study permitted the examination of multiple transmission sites. We chose a prospective design in order to avoid recall bias and examine separately risk factors for infections and risk factors disease. The analysis strategy was designed to estimate the size of the increased risk conferred by a particular factor. Because the number of non-infected individuals were so numerous, and the rates of infection and ATL relatively low, a nested case-control analysis proved most efficient.

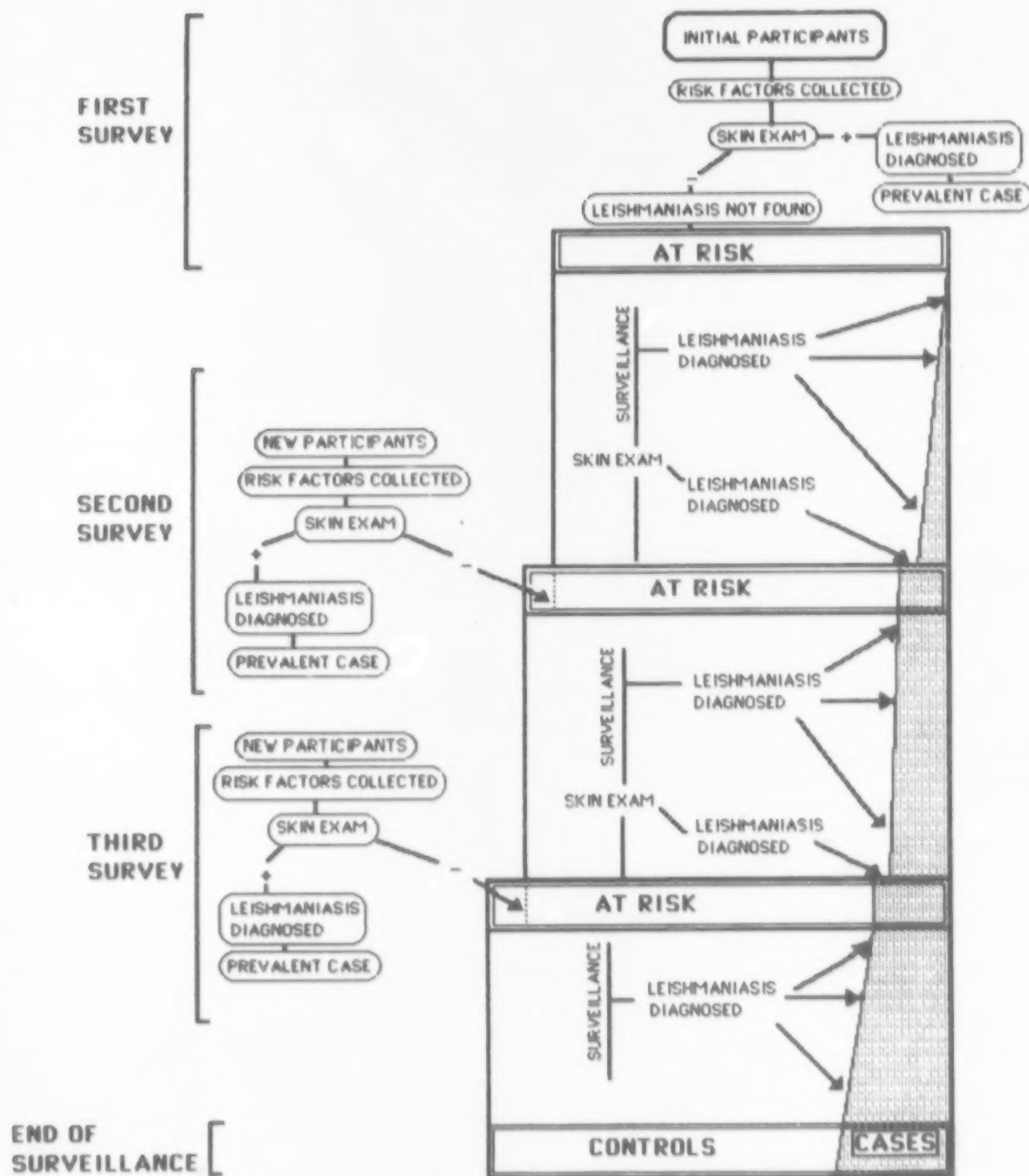
Methods

Study Design and Definitions: Cases of Leishmania infection and disease and their respective controls were drawn from the longitudinal study described above. Essentially two cohorts were defined, one for evaluation of risk factors for infection and another for risk factors for disease, as described in Figures 4 and 5. The evaluation of risk factors for infection was restricted to persons initially LST negative at the time of enrollment and who participated in two or three of the sequential surveys. Cases of infection were defined as persons who converted from an initially negative LST reaction to a positive LST during a later survey or diagnostic evaluation. Controls were randomly

STUDY DESIGN RISK FACTORS FOR INFECTION



STUDY DESIGN RISK FACTORS FOR TEGUMENTARY LEISHMANIASIS



chosen from those who remained skin test negative, matched with a case in the village of residence and year of skin test conversion. The cohort defined for the evaluation of risk factors for clinically apparent leishmaniasis excluded the 43 prevalent cases of leishmaniasis that were diagnosed during the enrollment survey. Incident cases of leishmaniasis, defined as described in the previous methods section, could meet either parasitological, clinical-lab, immunological, or likely diagnostic categories. Criteria for controls were: 1) Active diagnosis i.e., typical or suspect leishmanial lesions were not observed during the three serial physical exams nor during active surveillance; 2) They were skin tested during at least two of the three surveys and remained skin test negative; 3) Residence in the same village as the case; 4) Skin examination undertaken before and after the date on which the case was diagnosed. Three unique controls were randomly chosen for each clinically apparent case based on the preceding criteria. In total 89 participants were excluded from the control group because they presented typical Leishmania lesions that were not diagnostically confirmed.

Data collection: Instruments were developed, and pilot tested prior to the first survey. First, recognized and likely risk factors were listed. The study was discussed with community leaders and former leishmaniasis patients in the study area, to ascertain the daily habits of the residents and become familiar with local terms and phraseology regarding risk factors of interest. The questionnaire was prepared in Spanish and revised by repeated testing with native Colombians in Cali. Three instruments were employed to collect risk factor information:

- 1) The Individual Questionnaire concerning individual characteristics and behaviors was answered in person by each family member; mothers or immediate caretakers responded for minors.
- 2) A Household Questionnaire obtained information from the head of the household regarding risk factors common to the household. The head of household was defined as the person responsible for the economic support of the household. These two questionnaires were completed through personal interviews.
- 3) After observing and measuring the ecological and physical characteristics of the residence and its surrounding, this information was recorded on the Household Observation Form. The Household Questionnaire and Household Observation Form were completed during subsequent surveys for individuals who had moved to a new residence.

Data Management and Statistical Analysis: Data was entered using a defined screen format which included range checks and double entry features (Data Entry/SPSS). Frequency distributions of each potential risk factor were examined for cases and controls combined. Categories and recoding of variables were based on logical categories or distributions. Variables lacking sufficient variability were excluded from subsequent analyses. Initially, cases were compared to controls, by comparing frequencies of

categorical variables (Chi² test) or means of continuous variables (Student's T Test). The strength of the association between a risk factor and either Leishmania infection or disease was estimated by the odds ratio (OR) = odds of being a case given risk factor is present/odds of being a case given risk factor is absent. Variables likely to be associated with the case control status ($P < .15$) were evaluated in logistic regression models, which controlled for age and sex. To determine the amount of risk incurred beyond that related to farming, these likely risk factors were also assessed in models adjusted for sex, age, and farming occupation. Because the association between age and infection risk was not linear, age was included as a categorical variable for the adjusted analyses. Prevalence of risk factors was approximated by the distribution of the factor in the control group.

Results

Risk factors for infection: Table 7 demonstrates that older age, male gender, and years of residence were associated with leishmania infection. No association was observed between race and infection, however insufficient variability existed to evaluate race as a risk factor. Farming was a very prevalent risk factor; involving 33.5% of the controls. Overall being a farmer implied a nine fold increase in risk of infection (Table 8) and a 2.41 fold increase in risk compared to non-farmers of the same sex and age group (Table 11). A marked dose effect was observed between number of days of farming per week and infection. Certain farming activities appear to augment the risk associated with farming; in particular farmers who prepared for cultivation by clearing land of trees and brush incurred a greater risk of infection than farmers who did not clear land, O.R. = 13.36 versus 6.92, respectively. Although hunting was associated with infection (Crude O.R. = 4.3, adjusted O.R. = 2.91) this activity is infrequent; 6.2% of controls reported having hunted or accompanied someone hunting during the 12 months preceding enrollment. Lumbering was also an infrequent occupation, 3.5% of controls reported this exposure which is associated with Leishmania infection, (Crude O.R. = 4.66, adjusted OR = 2.36). Fishing activities were reported by 12.8% of controls and was associated with Leishmania infection to a moderate extent (crude O.R. = 2.36, adjusted O.R. = 1.56). Forest activities were frequently reported by both cases and controls and most were strongly associated with infection (Table 9). The factors displayed in Table 9 concern entering or passing through the forest for any reason, whereas specific reasons for entering the forest are evaluated in Table 10. For each additional hour spent in the forest the risk was increased by 1.26 (adjusted O.R., Table 11). Also being in the forest during non-daylight hours was associated with a marked increase in the risk of infection. (Entering forest after sunset, crude O.R. = 44.3; adjusted O.R. = 13.27). Residents of the study area entered the forest for many reasons besides farming, hunting, fishing, and lumbering (Table 10). Collection of wood and fruits increased the risk of infection associated with entering the forest but other forest related activities had no effect on the amount of risk associated with entering the forest. In some localities homes were surrounded by the forest such that all travel away from the homestead involved walking through the forest. For these individuals forest exposures were not separable. Adjusted

Table 7: RELATIONSHIP BETWEEN SOCIODEMOGRAPHIC CHARACTERISTICS
AND *L. braziliensis* ssp INFECTION*

CHARACTERISTICS	CASES		CONTROLS		UNADJUSTED	
	N=227	%	N=227	%	O.R.	(95% C.I.)
Age (years) ¹						
<10.0 - No. (%)	15	(7)	109	(48)	1	
10.0 - 29.9	82	(36)	76	(33)	7.8	(4.2-16)
≥30	130	(57)	42	(18)	22	(11.8-43)
Age (years) ¹	35.8 ± 19.9		16.9 ± 18.7		N.A.	N.A.
Male Sex - No. (%) ₂	138	(61)	107	(47)	1.7	(1.20-2.5)
Race						
Black or Indian - No. (%)	213	(94)	215	(95)	1.0	
Mixed and Caucasina-No. (%)	14	(6)	12	(5)	1.2	
Length of residence ¹ in study area (years)						
<1 No. (%)	19	(8)	34	(15)		
1 - 5.9	40	(18)	83	(37)		
6 - 10.9	32	(14)	46	(20)		
11- 20.9	57	(25)	32	(14)		
> 20	79	(35)	30	(13)		
unknown	0	(0)	2	(1)		
Length of residence ¹ in study area (years)	4.4 ± 5.4		3.4 ± 6.4			

± = Indicates standard deviation

1 = P<0.001

2 = P<0.01

* = Frequency distribution compared by Chi² test, means compared by student's T test

Table 8: RELATIONSHIP BETWEEN FARMING ACTIVITIES
AND *L. braziliensis* ssp INFECTION*

ACTIVITY	CASES N= 227		CONTROLS N=227		Unadjusted O.R.
	No.	(%)	No.	(%)	
Farming occupation: Yes ¹	187	(82)	76	(33)	9.3 ²
No**	40	(18)	151	(66)	1.0
Cleared land of trees in last year ¹	117	(51)	33	(14)	13.4
Did not clear land of tree in last year	70	(31)	42	(18)	6.9
Planted cocoa in last year ¹	109	(48)	34	(15)	12.1
Did not plant cocoa in last year	77	(34)	42	(18)	6.9
Planted crop other than cocoa ¹	156	(69)	55	(24)	10.7
Only plated cocoa	31	(14)	21	(9)	5.4
Worked on additional farms ¹	142	(63)	58	(26)	10.1
Only worked own farm	43	(19)	16	(7)	9.2
Went to a farm in last year ¹	218	(96)	156	(69)	11.0 ³
Did not go to a farm in last year	9	(4)	71	(31)	1.0
Goes to the farm every day ¹	17	(7)	1	(0)	134.1
4-6 days per week	93	(41)	42	(18)	17.5
1-3 days per week	85	(37)	72	(32)	9.3
less than weekly	23	(10)	41	(18)	4.4
Never in last year	9	(4)	71	(31)	1.0

* = Frequency distributions compared by Chi² test

** = Reference category for farming activities

1 = P<0.001

2 = 95% C.I. (OR) = 5.99 - 14.41

3 = 95% C.I. (OR) = 5.14 - 24.41

Table 9: RELATIONSHIP BETWEEN ENTERING FOREST
AND *L. braziliensis* ssp INFECTION

ACTIVITY***	CASES N= 227		CONTROLS N=227		Unadjusted O.R.
	No.	(%)	No.	(%)	
Entered forest frequently ¹	190	(84)	89	(39)	33.4
infrequently	34	(15)	91	(40)	5.8
never**	3	(1)	47	(21)	1.0
Hours per day in the forest: >7 ¹	107	(47)	22	(10)	76.2
5 - 7.0	60	(26)	50	(22)	18.8
0.1 - 4.9	57	(25)	108	(48)	8.3
Entered forest before dawn ¹	38	(17)	7	(3)	85.0
Entered forest but not before dawn	186	(82)	173	(76)	16.8
Entered forest after sunset ¹	79	(35)	26	(11)	47.6 ²
Entered forest but not after sunset	145	(64)	154	(68)	14.7 ³
Entered forest at night ¹	17	(7)	6	(3)	44.4
Entered forest but not at night	207	(91)	174	(77)	18.6
Hours per day in the forest ¹	6.2 + 2.4		4.0 + 2.3		1.5 ⁴

* = Frequency distributions compared by Chi² test, means compared by student's test

** = Reference category for forest activities

*** = Reported for prior 12 months

1 = P<0.001

2 = 95% C.I. (OR) = 13.66 - 165.90

3 = 95% C.I. (OR) = 4.49 - 48.44

4 = O.R. per hour in forest, 95% C.I. = 1.36-1.60

Table 10: RELATIONSHIP BETWEEN SPECIFIC FOREST ACTIVITIES
AND *L. braziliensis* ssp INFECTION*

ACTIVITY**	CASES N= 227		CONTROLS N=227		Unadjusted O.R.
	No.	(%)	No.	(%)	
Entered forest to obtain water ¹	91	(40)	69	(30)	20.7
Entered forest, but not to obtain water	133	(59)	111	(49)	18.8
Did not enter the forest***	3	(1)	47	(21)	1.0
Entered forest to collect wood ¹	164	(72)	79	(35)	32.5
Entered forest but not to collect wood	60	(26)	101	(44)	9.3
Entered forest to gather fruits	52	(23)	28	(12)	29.1
Entered forest but not to gather fruits	172	(76)	152	(67)	17.7
Entered forest to visit relatives or friends ¹	127	(56)	100	(44)	19.9
Entered forest, but not to visit relatives or friends	96	(43)	80	(35)	13.5
Entered forest to travel to another town ¹	116	(51)	77	(40)	23.6
Entered forest but not to travel to another town	108	(48)	103	(45)	16.4
Entered forest to travel to school ¹	19	(8)	10	(4)	29.8
Entered forest but not to travel to school	205	(90)	170	(75)	18.9
Entered forest to defecate	87	(38)	68	(30)	20.0
Entered forest but not to defecate	137	(60)	112	(49)	19.2
Entered forest to bathe	50	(22)	41	(18)	19.1
Entered forest but not to bathe	174	(77)	139	(61)	19.6

* = Frequency distributions compared by Chi₂ test

** = Activity during preceeding 12 months

*** = Reference category

1 = P<0.001

Table 11: Relationship between selected individual characteristics and behaviors and *L. braziliensis* infection, after adjustment.

Variable	Age and sex adjusted		Age, sex, farming adjusted	
	O.R.	(95% CI)	O.R.	(95% CI)
Male sex	1.9 ¹	(1.2 - 2.9)	1.3	(0.8 - 2.2)
Age (years):				
< 10.0	1.0 ¹		1.0 ¹	
10 - 29.9	7.9	(4.2 - 14.9)	4.5	(2.2 - 9.2)
> 30.0	23.3	(12.2 - 44.7)	9.3	(4.0 - 21.7)
Farming occupation	2.8 ¹	(1.5 - 5.2)	2.8 ¹	(1.5 - 5.2)
Hunting*	2.4 ³	(1.2 - 4.9)	2.0	(1.0 - 4.1)
Lumbering*	2.4	(1.0 - 5.7)	2.0	(0.8 - 4.7)
Fishing*	1.6	(1.0 - 2.7)	1.4	(0.8 - 2.4)
Hours per day in forest*	1.3 ³	(1.1 - 1.4)	1.2 ¹	(0.4 - 3.6)
Entered forest after sunset	13.3 ¹	(3.4 - 51.2)	11.5 ¹	(2.8 - 44.8)
Entered forest, but not after sunset	6.8	(1.9 - 23.3)	6.8	(1.9 - 24.4)

* = Activity reported for preceding 12 months.

1 = P < .001.

2 = P < .01.

3 = P < .05.

Table 12: Relationship between ecological, environmental conditions, and *L. braziliensis* infection*.

Condition	Cases N = 227		Controls N = 227		Unadjusted O.R. (95% CI)
	No.	%	No.	%	
Tall trees within 10m of residence ³	129	(57)	104	(46)	1.6 (1.1 - 2.2)
Trees with exposed roots within 10m of residence ³	76	(33)	53	(23)	1.6 (1.1 - 2.5)
Tall trees, surrounding farm (yes) ³ and visited farm in last 12 months	170	(78)	134	(59)	2.1 (1.4 - 3.1)
No swamp within 10m of residence ³	171	(75)	152	(67)	1.5 (1.0 - 2.3)
Distance from next house > 15m ³	115	(51)	92	(41)	1.5 (1.0 - 2.1)
Distance from next house (meters)**	37.8 ± 103		31 ± 32		N.A.
Distance of floor above ground = 0.3-1m (reference < .3m or > 1) ²	133	(59)	100	(44)	1.8 (1.2 - 2.6)
Distance of floor above ground (meters)	0.6 ± .41		0.6 ± .48		N.A.
<u>Wall construction:</u>					
Wood, bricks, or zinc	87	(38)	100	(49)	1.0
Wood and bamboo	33	(14)	33	(14)	1.1
Bamboo or thatch	107	(47)	94	(41)	1.3
<u>Floor material:</u>					
Dirt, cement, or wood	185	(81)	198	(87)	1.0
Palm, bamboo, other open	42	(18)	29	(13)	1.5 (0.9 - 2.6)
<u>Roof material:</u>					
Tile, zinc, carton, closed	117	(52)	147	(65)	1.0
Thatch	109	(48)	78	(35)	1.8 (1.2 - 2.6)

* = Frequency distributions compared by Chi² test, means by Students t-test.

** = 1. P < .001.

2 = P < .01.

3 = P < .05.

Table 13: Relationship between ecological, environmental conditions and *L. braziliensis* infection, after adjustment.

Variable	Age and sex adjusted		Age, sex, farming adjusted	
	O.R.	(95% CI)	O.R.	(95% CI)
Tall tree within 10 m of residence	1.7 ²	(1.1 - 2.6)	1.7	(1.1 - 2.6)
Trees with exposed roots within 10 m of residence	1.7 ²	(1.1 - 2.8)	1.7	(1.0 - 2.7)
Tall trees surrounding farm	0.8	(0.4 - 1.3)	0.7	(0.4 - 1.3)
No swamp within 10 m of residence	1.6	(1.0 - 2.6)	1.6 ²	(1.0 - 2.7)
Distance from next house > 15 m)	1.5	(0.9 - 2.3)	1.5	(0.9 - 2.3)
Distance of floor above ground 0.3-1.0 m	1.6 ²	(1.0 - 2.5)	1.6	(1.0 - 2.5)
Floors bamboo, palm, open	1.2	(0.6 - 2.1)	1.1	(0.6 - 2.1)
Roof thatch, palm leaves	2.0 ¹	(1.3 - 3.1)	2.0 ¹	(1.3 - 3.2)

1 = P < .01.

2 = P < .05.

Table 14: RELATIONSHIP BETWEEN SOCIODEMOGRAPHIC CHARACTERISTICS
AND TEGUMENTARY LEISHMANIASIS

Characteristics	CASES N = 34		CONTROLS** N = 102		Unadjusted O.R.
	No.	(%)	No.	(%)	
Age (Years) ¹					
<10.0	4	(11.8)	48	(47.1)	1.00
10.0 - 29.9	20	(58.8)	35	(34.3)	6.86
> 30	10	(29.4)	19	(18.6)	6.32
Age (Years) ³ mean $\pm$ s.d. ¹	23.6 $\pm$ 17.1		16.3 $\pm$ 17.5		
Male Sex ³	25	(73.5)	55	(53.9)	2.57
Race					
Black or Indian	31	(91.2)	99	(97.1)	1.00
Mixed of Caucasian	3	(8.8)	3	((2.9)	3.10
Length of residence (years)					
In study area	14.3 $\pm$ 14.9		9.3 $\pm$ 12.2		N.A.

* = Frequency distributions compared by Chi² test means by student's test

** = lacked infection and any suspect lesions

1 = P<0.001

2 = P<0.01

3 = P<0.05

Table 15: RELATIONSHIP BETWEEN OCCUPATIONAL AND BEHAVIORAL FACTORS AND TEGUMENTARY LEISHMANIASIS*

FACTOR		CASES N = 34		CONTROLS N = 102		UNADJUSTED O.R.
		No.	(%)	No.	(%)	
Farming occupation	yes ¹	27	(79)	35	(34)	7.4
	no	7	(21)	67	(66)	1.0
Hunting***	yes	5	(15)	14	(14)	1.1
	no	29	(85)	88	(86)	1.0
Lumbering***	yes ³	5	(15)	3	(3)	5.7
	no	29	(85)	99	(97)	1.0
Fishing***	yes ³	11	(32)	15	(15)	2.8
	no	23	(68)	87	(85)	1.0
Hours per day in forest*** ¹ mean $\pm$ s.d.		59 $\pm$ 2.4		4.3 $\pm$ 2.5		N.A.
Entered forest after sunset*** ²		11	(32)	15	(15)	24.9
Entered forest but not after sunset		23	(68)	70	(69)	11.2
Dented entering forest		0	(0)	17	(17)	1.0
Visited a farm***	yes ²	32	(94)	73	(72)	6.4
	no	2	(6)	29	(28)	1.0

* = Frequency distributions compared by Chi² test, means by Student's test

** = lacked infection and any suspect lesions

*** = Activity reported for preceding 12 months

1 = P<0.001

2 = P<0.01

3 = P<0.05

Table 16: RISK FACTORS FOR PATHOGENECITY

VARIABLE	DISEASE*		INFECTED**		UNADJUSTED ODDS RATIO
	N = 27 No.	(%)	N = 207 No.	(%)	
Highly endemic village	13	48.1	81	39.1	1.52
Moderately endemic village	8	29.6	69	33.3	1.10
Low endemic village	6	22.2	57	27.5	1.00
Age (Years) ¹					
<10	4	14.8	15	7.2	4.57
10.0 - 29.9	16	59.3	72	34.8	3.81
> 30	7	25.9	120	58.0	1.00
Age (Years) mean $\pm$ s.d.	22.3 $\pm$ 16.3		36.25 $\pm$ 20.3		
Farming occupation					
yes	20	74.1	168	81.2	0.66
no	7	25.9	39	18.8	1.00
Went to a farming last year					
yes	25	92.6	199	96.1	0.50
no	2	7.4	8	3.9	1.00
Hunting in last 12 months					
yes	4	14.8	48	23.2	0.58
no	23	82.5	159	76.8	1.00
Fishing in last 12 months					
yes	9	33.3	49	23.7	1.61
no	18	66.7	158	76.3	1.00
Lumbering in last 12 months					
yes	5	18.5	26	12.6	1.58
no	22	81.5	181	87.4	1.00
Goes to farm every day	2	7.4	17	8.2	2.71
Goes to farm 1-6 days per week	22	81.5	159	76.8	3.18
Goes to farm less than weekly	1	3.7	23	11.1	1.00
Cleared land of trees					
yes	14	51.9	102	49.3	0.76
no	6	22.2	66	31.9	0.51
Doesn't farm	7	25.9	39	18.8	1.00

* = Diagnosed cases of leishmaniasis initial. LST negative

** = Cases of infections without leishmaniasis of active lesions

¹ = p < 0.01

analyses for sociodemographic and occupational risk factors are displayed in Table 14. In general, associations observed by crude analyses persisted after adjustment for age and sex, although the strength of associations decreased. In addition, we adjusted by sex, age and farming occupation but found that controlling for farming changed the O.R. for various other occupations and behaviors very little. Hence, it appears that risks were conferred by activities such as entering the forest were independent of farming status.

Evaluation of risk factors obtained from the Household Questionnaire and Household Observation Form are summarized in Tables 12 and 13. Much of the information and the associations obtained through the household questionnaire duplicated that observed using the individual questionnaire. The importance of farming as a risk factor for infection was verified. However, most characteristics of the families' farm such as crops grown, size of farm and ecological conditions surrounding the farm were not associated with an increased risk of infection. In these analyses all persons were assigned the exposure characteristic of their household farm regardless of whether the individuals actually participated in agricultural activities, thereby misclassifying actual exposure. Also, insufficient variability was present to assess the effect of many exposures. The presence of large trees, or trees with exposed roots nearby residences increased risk of infection moderately.

Risk Factors for Tegumentary Leishmaniasis: Risk factors for leishmanial lesions resembled those observed for infection, Tables 14 and 15. Due to the smaller number of cases, estimates of the risk associated with infrequent activities, such as hunting and lumbering, are imprecise. No factors were associated with disease that had not been associated with infection.

To determine whether there were any risk factors associated with pathogenicity, we utilized an additional case control design. Cases of leishmaniasis who were initially LST negative at enrollment were compared to "controls" consisting of persons who LST converted without developing clinical manifestations. To avoid misclassifications, persons with any suspect of diagnosed skin lesion were excluded from these controls. Young age appeared to be associated with an increased pathogenicity (Table 16). This parallels the observation that the maximal incidence of infection was between 40 and 60 years of age whereas the highest illness rate was observed between 10 and 29 years of age. Gender did not impact on pathogenicity, nor did environmental and behavioral factors related to vector contact.

Discussion

The major risk factors for both Leishmanis infection and disease and occupations are behaviors that presumably increase contact with the forest habitat of Leishmania laden sandflies. The increased risk associated with increasing age and male gender are consistent with finding of others (15,18,19) and are probably due to the occupations, farming, hunting, etc. that predominate in males and adulthood. A clear dose response

was observed with hours spent in the forest. In contrast to observations in Guatemala (19) we observed that activities that lead to presence in the forest after nightfall were especially hazardous. These findings indicate the types of behavioral intervention that may be most appropriate to this setting. Although the most common risk factor, farming, is not amenable to intervention, other activities, such as hunting and fishing, could be curtailed. Avoidance of the forest during night could be encouraged and may also impact on other vector-borne diseases such as malaria. Complete avoidance of the forest is obviously impossible in these communities due to the multiple forest related activities encountered. However, the dose response relationship that we observed would predict that even a reduction in forest activities would prove beneficial.

The second application of the findings is to identify the individuals for whom both behavioral and vector-control measures would prove most beneficial. Clearly adult male agricultural workers who spend long hours in the forest and need to enter the forest after nightfall could be targets for self-protection measures such as repellents, repellent soaps, or repellent clothing (3). The indicators for insecticides are less clear. Nearly all homes report insecticide spraying, so we could not determine whether insecticides reduced domestic transmission. Insecticide spraying and forest clearing in the periphery of farms may also reduce risk associated with farming. The observation that similar risk factors were found for infection and disease may indicate that the host is the major determinant of pathogenicity. This hypothesis is supported by the increased pathogenicity observed in younger individuals.

Summary - Implications for Control

Studies of human *L. braziliensis* ssp infection in the municipality of Tumaco, Narino have yielded a wealth of information about the natural history of leishmaniasis caused by this species complex, and the methodologic bases for similar studies in other foci of transmission. Incidence, prevalence and risk factors for infection and disease were defined for the inhabitants of 15 villages within a focus of transmission of primarily *L.b. panamensis*, and *L.b. braziliensis*. Such information on disease burden is essential to the development and implementation of strategies for the reduction and prevention of leishmaniasis.

Among the communities studied, prevalent infection and disease were the source of a large portion of the morbidity recorded over the three years of observation. Thus, many incident cases of leishmaniasis derived from already infected individuals. Infection was more common than disease with an overall ratio of 10:1. Prevalent disease exceeded incident disease over the three years of observation, reflecting the accumulation of both infection and disease over years past. The incidence of illness was correlated with the rate of infection in each village, indicating a relatively constant rate of pathogenicity throughout the study area. In foci such as these, secondary prevention of the progression of disease and infection to disease, among the individuals at risk, is an important consideration in reducing the disease burden. Chemotherapy and immunotherapy might

be indicated, particularly in more highly endemic settings, in order to control disease manifestations in previously infected individuals.

Risk factors for infection and disease were found to be the same: occupational and/or behavioral exposure to the forest; male gender; and increasing age up to 30 years, at which time risk reached a plateau. Risk associated with contact with the forest was clearly dose dependent, increasing with the number of hours spent in the forest. Activities undertaken in the forest after nightfall were particularly hazardous. Pathogenicity was greatest among younger individuals with the highest incidence rate for disease being observed between 10 and 29 years of age. In this or similar foci, potential preventive interventions include reduction of exposure to the forest especially nighttime incursion, use of some form of repellent by male agricultural workers, and clearing of the forest around farms.

Based on these observations, reports of others, and WHO recommendations it appears that control strategies must be tailored to the epidemiology of ATL in a given community. Three major considerations are 1) the relative role of new infections or pre-existing infections in disease burden; 2) Site (S) of transmission; 3) High risk populations. Where numerous chronic or recurrent cases are found attention to efficient surveillance, diagnosis and case management is appropriate. Where intra-domestic transmission occurs, insecticide impregnated curtains or bednets may reduce transmission. Peridomestic transmission could be reduced by reservoir control, perimeter clearing and possibly insecticide spraying. Where risk is concentrated in agricultural settings personal protection measures, perimeter spraying or clearing and education regarding riskier circumstances should be evaluated. For high risk occupational groups such as road construction workers or lumbermen personal protection and risk reduction education may prove beneficial. None of the above mentioned measures have been evaluated sufficiently to warrant their wide adaptation. However pilot intervention trials that measure efficacy, cost to benefit ratio and community acceptance are needed. This project has developed methodologies and an awareness of the design issues inherent in such trials.

The selection of communities for intervention trials is quite challenging. Community interest and collaboration, a pre-existing primary care infrastructure and community leadership and cohesion could facilitate such trials. The rates of end points to be impacted upon, such as sandfly biting, *leishmania* infection, and leishmaniasis cases must be high, otherwise sample size and the length of the trial will be prohibitive. Logistical consideration and the support of local health care providers are integral to planning such trials. The observation that incidence rates are highest in sparse settled forested areas indicates logistical planning is crucial to such trials.

Another important consideration for these intervention trials is the selection of an appropriate design. Clearly the results of randomized trials are often less ambiguous than those of a before and after study or interventions in "matched" settings. Quasi-

experimental designs may falter if year-to-year rates of leishmania transmission are quite variable. The unit of randomization, such as community, household or individual must also be considered carefully. Each unit has inherent advantages and disadvantages.

Because we and others (15,18,19) have observed relatively low incidence rates of ATL, the use intermediate endpoints of interventions such as sandfly counts, biting counts, and LST conversion may provide more rapid answers to comparisons of intervention methods. Once interventions are demonstrated to effect intermediate endpoint, study of their impact on new cases of ATL would be appropriate.

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Risk Factors Associated with Cutaneous Leishmaniasis Infection and Disease in the State of Campeche, Peninsula of Yucatan, Mexico

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Introduction

In 1987 a review of knowledge regarding Cutaneous Leishmaniasis (Chiclero's Ulcer) in Mexico was presented (1). It can be summarized as follows: Seidelin in 1912 described the disease at the Peninsula of Yucatan and named it "Chiclero's ulcer"(2); Shattuck in 1933 (3), Beltrán & Bustamante in 1942 (4) and Martínez in 1951 (5) reported cases in the same geographic area; Biagi et al. working at the same endemic area from 1953 to 1965 carried out studies regarding clinical picture, histopathological pattern, geographical distribution, parasite and vector identification (6-10).

However, there is a complete absence of knowledge about reservoirs in Mexico. In summary the parasite responsible of Mexican Localized Cutaneous Leishmaniasis (Mex LCL), well known as Chiclero's Ulcer, has been identified as Leishmania mexicana mexicana (11,12); and the only proven vector identified is Lutzomyia olmeca olmeca (13,14). Lainson and Strangways-Dixon in 1962 working at Belize indentified Ototylomys phyllotis, Heteromys desmarestianus, and Nictomys sumichrasti as animal reservoirs of Leishmania mexicana mexicana (15). On the basis of this limited knowledge it was considered justified to initiate a comprehensive study of leishmaniasis in the Peninsula of Yucatan, Mexico.

The general objective of the Phase I project (1987-89) was to assess the relative importance of Mexican Localized Cutaneous Leishmaniasis (Mex LCL) in an endemic area of Mexico, the State of Campeche, Peninsula of Yucatan, through the development of diagnostic measures as a first step in the control of disease both in the individual and in the community. Specific objectives were: 1) to assess the incidence of leishmaniasis; 2) to determine sensitivity and specificity of the ELISA assay for IgG antibodies to L. mex. mex.; 3) to evaluate safety and efficacy of treatment of Mex LCL with meglumine antimoniate.

Upon completion of the Phase I project, a Phase II project (1990-92) was undertaken to study risk factors associated with acquisition of infection and/or disease. Its specific objectives are: 1) to identify risk factors associated with acquisition of

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infection and/or disease in a population living in the endemic area; 2) to identify potential sandfly vectors; 3) to identify parasites isolated from human cases; and 4) to determine sensitivity and specificity of the ELISA assay for IgM antibodies to L. mex. mex.

Materials & Methodology

Phase I, 1987-89 studies:

In order to obtain reliable knowledge about the incidence of Mex LCL in the State of Campeche, we interviewed and performed skin tests (Montenegro intradermal test) on a sample of 449 persons randomly selected from men aged 15-45 years, from 7 Rural Health Posts (RHP)² (16). Diagnosis of cases detected from January to December 1987 was established based on clinical examination, parasitological demonstration (smear, isolation-culture, biopsy) when possible, histopathology (to exclude other pathology) and immunological tests, skin-test and ELISA (17,18).

Two-hundred and twenty-three sera samples were assayed to assess the sensitivity and specificity of ELISA for IgG antibodies to L. mex. mex. (A = 75 sera samples from healthy negative skin-test; B = 56 from healthy positive skin-test; C = 74 from patients with Mex LCL; and D = 18 from patients suffering other diseases). ELISA for IgG was carried out according to El Amin et al. (19) employing a promastigote suspension of 1×10^7 /ml and a goat anti-IgG heavy chain conjugated to peroxidase (SIGMA A6029) diluted 1/1000 in PBS-T. Absorbances were read at 490nm. Results were determined as positive or negative according 2x2 Table and Chi-square test (20).

In order to evaluate the safety and efficacy of treatment a total of 53 males ranging in age from 8 to 75 years (average age 26 years) were treated for Mex LCL with meglumine antimoniate during 1987-1988. Diagnosis was established as described (17-19). Criterion for the application of treatment followed those established by CHEMLEISH/WHO (21).

Phase II, 1990-92 studies:

To identify risk factors associated with the acquisition of infection and/or disease the study was designed as a "case control" study including three "controls" per "case". A total of four "ejidos" (small village) were selected to carry out this study. A questionnaire including age, sex, occupation(s), time of living in the "ejido", workplace, home-working-play distance, going or not going to the field, household living conditions, house location, house building materials and protective measures against insects was conducted as was a skin-test. A complete clinical history, parasitological and immunological diagnosis test were done in all "cases" detected.

² These were located in the isthmus with a tropical climate, humidity of over 80% and an annual average rainfall of over 1400mm.

La Libertad, a small village with a total of 628 inhabitants located at 24 km from Escarcega (Biagi's studies area) and with 12 "well defined" TDR/WHO/LEISH criterion "cases" during 1989-90 was chosen as the study area for the identification of potential sandfly vectors of *Leishmania* sp. The methodology for sandfly sampling included: 1) Searching resting sites approximately 1km surrounding the "ejido"; 2) Use of tent traps (Shannon) for all-night anthropophilic collections; and 3) Use of CDC light traps in sylvatic areas without air movements between 18:00 to 21:00 hours. This study was conducted in the surrounding area of La Libertad from October 1990 to September 1991. Taxonomic classification was completed according established methodology (22-23).

Identification of parasites isolated from human cases was achieved through isolation, culture, and maintenance on Senekje's biphasic medium, hamster inoculation, cryopreservation and isoenzyme characterization by cellulose acetate electrophoresis employing six enzymes (NH, GGPDH, PGM, GPI, MPI, GPGDH) according to established methodology (25-26).

To determine the sensitivity and specificity of the ELISA assay for IgM antibodies to *L. mex. mex.* a total of 79 sera were collected. These consisted of: Group I - 40 samples from healthy negative skin-test patients; and Group II - a) 21 samples from patients with Mex LCL parasite-positive demonstration and IgG anti-*L. mex. mex.* ELISA positive and b) 9 samples from patients parasite-positive and IgG negative and 9 from patients parasite-negative demonstration and IgG ELISA positive, all of them with less than 12 months of clinical evolution. ELISA was carried out according El Amin et al. (19) employing a promastigote suspension at 1×10^7 /ml. and a goat anti-IgM heavy chain conjugated to peroxidase diluted 1/1000 in PBS-T. All sera were treated with 2-ME to expose IgM and eliminate IgG (28). Absorbances were read at 490 nm. Results were considered positive when an absorbance value was more than 0.374 using a 1/16 dilution, this was the mean plus 2 SD at Group I control sera (29).

Results

Phase I, 1987-89 studies:

A positivity rate of 24-90% (mean 43%) for Mex LCL was found in the seven Rural Health Posts studied (Table 1). Furthermore, 72 patients were examined from January to December 1987 and 56 of them acquired the disease in 1987. Therefore, we found amongst men aged 15-45 years in the focus area an annual incidence rate of 508 per 100,000 inhabitants (16).

The ELISA results for the four groups of sera are shown in Table 2. A total of 56 of the 74 sera from patients with Mex LCL, gave positive reactions (i.e. 76% sensitivity) of a titre of 1:40 and the positivity rate was lower in patients with long-standing infections. Table 3 shows differences in reactivity considering parasite positivity and time of evolution (17).

Table 1. Montenegro skin test positivity in seven rural communities in Campeche, Mexico

Rural community	No. of subjects	No. positive
Constitucion	80	46 (57%)
Don Samuel	31	28 (90%)
A. Obregon	81	20 (24%)
Yohaltun	30	15 (50%)
Centenario	131	33 (25%)
Carmen II	33	20 (60%)
R. Payro	63	34 (53%)
Total	449	196 (43%)

Table 2. Enzyme-linked immunosorbent assay results for the different groups of sera tested.

Group	No. of sera	No. positive	Percentage reactivity
A. Healthy human beings M-a	75	0	0
B. Healthy human beings M+b	56	4	7
C. Patients with leishmaniasis	74	56	76
D. Patients with other diseases	18	6	33

aM- = negative Montenegro test.

bM+ = positive Montenegro test.

Table 3. Enzyme-linked immunosorbent assay results for sera from patients with chiclero's ulcer according to parasitological diagnosis and stage of development

Patients	No. of sera reactivity	No. positive	Percentage
Acute p+a	34	31	91
Acute p-b	16	12	75
Chronic	24	13	54

aP+ = Parasites demonstrated

bP- = Parasites not demonstrated

A total of 42 patients with Mex LCL completed their course of treatment of with meglumina antimoniate. From these, three cases were classified as "acute" (i.e. less than a year of clinical infection), and 10 as "chronic" (i.e. 2 to 38 years from onset). The response to treatment in both groups is shown in Tables 4 and 5. Importantly, Table 5 shows that when systemic treatment was given to both "acute" and "chronic" cases, no significant difference in the results, regardless average doses, was observed (27).

Table 4. Response to Treatment depending on via administration with Meglumine Antimoniate in patients with Mex L.C. Leishmaniasis

Time of Evolution	Systemic		Via Intralesional		Total	
	Cured	Failure	Cured	Failure	Cured	Failure
Acute (n=32)	25 (90%)	3 (10%)	3 (75%)	1 (25%)	28 (88%)	4 (12%)
Chronic (n=10)	7 (88%)	1 (12%)	2 (100%)	0 (0%)	9 (90%)	1 (10%)
Total	32 (89%)	4 (11%)	5 (84%)	1 (16%)	37 (88%)	5 (12%)

Table 5. Mex LCL Response to Treatment with Meglumine Antimoniate. Average doses in cured patients

Time of Evolution	Dose (No. of ampules)	
	Systemic Range (average)	Intralesional Range (average)
Acute	8-33 (23)	5-9 (5)
Chronic	10-30 (22)	13-18 (15)

Phase II, 1990-92 studies:

It was possible to detect 58 well defined "cases" of Mex LCL according TDR/WHO/LEISH (i.e. parasitological demonstration positive) in the four "ejidos" selected for the study and 213 "controls" from the 2,907 total population of inhabitants who were studied (questionnaire and skin-test). In Table 6 the main risk factors associated with acquisition of infection and/or diseases identified after all statistical data analysis are shown. From all data analysed we can conclude that the most important risk factor is "exposure" and as a consequence age, sex, occupation, working place and going to the field are closely related.

Table 6. Risk factors associated with acquisition of infection and/or diseases in an endemic area of Mex CLC.

Risk Factor	No. of Cases (%)	No. of Controls (%)	Average	p ¹	Odds Ratio
Age:					
> 15 years	47 (81)	106 (49.8)	16.88	0.00	4.31
< 15 years	11 (19)	107 (50.2)			
Sex:					
Male	52 (89.7)	78 (36.6)	49.27	0.00	15
Female	6 (10.3)	135 (63.4)			
Going to Field*:					
Yes	55 (94.8)	97 (45.5)	42.98	0.00	21.92
No	3 (5.2)	116 (54.5)			
Skin test (IDR):					
Positive	34 (87.2)	20 (9.4)	113.9	0.00	65.62
Negative	5 (12.8)	193 (90.6)			

*Going to Field: Yes, going often and for sometime; No, not going to the field.

A total of 362 (100%) Lutzomyia sp. were collected employing the methodology described previously. Table 7 presents the frequency of species collected by sex. In Table 8 it is possible compare the different sandfly sampling methods used throughout one year (the CDC light traps were the best). Of the 169 (46.6%) Lutzomyias collected 137 (81%) were females. It is important to point out that only one of them was Lutzomyia olmeca, whereas 96 (56.8%) were L. deleoni and 26 (15.4%) were L. carpenteri. Searches of resting sites yielded 133 (36.7%) sandflies however, only 26 (19.5%) were females and only L. deleoni, L. cayennensis, L. shannoni and L. cruciata were found. Shannon traps captured 60 (16.6%) sandflies and 54 (90%) were females including L. cruciata 39 (65%), L. olmeca 5 (8.3%), L. deleoni, 5 *8.3%) and L. panamensis 3 (5.0%), but were not relevant as results.

Regarding anthropophilic activity by all-night collections employing Shannon traps, the highest activity was observed from 18:00 to 22:00 hours with a peak at 19:00 hours, and L. cruciata was the most anthropophilic species followed by L. olmeca and L. deleoni.

Table 7. Number of Lutzomyia species collected by sex (% in brackets)

Species	Male	Female	Total
<u>L. deleoni</u>	104 (28.7)	115 (31.8)	219 (60.5)
<u>L. cruciata</u>	2 (0.5)	47 (12.9)	49 (13.5)
<u>L. carpenteri</u>	14 (3.9)	27 (7.4)	41 (11.3)
<u>L. panamensis</u>	9 (2.5)	6 (1.6)	15 (4.1)
<u>L. shannoni</u>	7 (1.9)	4 (1.1)	11 (3.0)
<u>L. cayennensis</u>	6 (1.6)	5 (1.4)	11 (3.0)
<u>L. olmeca</u>	0	6 (1.6)	6 (1.7)
<u>L. gomezi</u>	1 (0.2)	2 (0.6)	3 (0.8)
<u>Ph. permirus</u>	0	3 (0.8)	3 (0.8)
<u>L. ovallesi</u>	2 (0.6)	0	2 (0.6)
No identification	2 (0.6)	0	2 (0.6)
Total	147 (40.6)	215 (59.4)	362 (100%)

Table 8. Number of *Lutzomyia* sp. caught (% in brackets) employing three different collection methods.

Month	Shannon	C.D.C	Resting Sites	T o t a l
Oct. 90	3 (0.8)	3 (0.8)	12 (3.3)	18 (5.0)
Nov. 90	2 (0.5)	9 (2.5)	12 (3.3)	23 (6.4)
Dec. 90	4 (1.1)	9 (2.5)	14 (3.9)	27 (7.5)
Jan. 91	6 (1.7)	36 (9.9)	36 (9.9)	78 (21.5)
Feb. 91	15 (4.1)	60 (16.6)	22 (6.1)	97 (26.8)
Mar. 91	18 (5.0)	26 (7.2)	0	44 (12.1)
Apr. 91	2 (0.5)	1 (0.3)	6 (1.7)	9 (2.5)
May. 91	0	0	2 (0.5)	2 (0.5)
Jun. 91	0	3 (0.8)	8 (2.2)	11 (3.0)
Jul. 91	5	10 (2.8)	3 (0.8)	18 (5.0)
Aug. 91	0	1 (0.3)	3 (0.8)	4 (1.1)
Sep. 91	5 (1.4)	11 (3.0)	15 (4.10)	31 (8.6)
	60 (16.6)	169 (46.6)	133.(36.7)	362 (100%)

Phase II, 1990-92 studies:

Studies for the identification of parasites isolated from human cases only began in June 1991 due to equipment and chemical reagents obtained during first semester 1991. However, it has been possible since January 1990 to date, isolate and maintain a total of 85 stocks from patients with a Mex LCL. Cryopreservation and isoenzyme characterization by cellulose acetate electrophoresis employing 6 enzymes mentioned previously, has been standarized. We have recently identified 16 strains and all of them are L. mex. mex.

The ELISA results for groups of sera studied are shown in Table 9. It is possible to observe that 22/39 (56%) cases were positive at titres > 1:16. Therefore, employing the methodology described previously, we have obtained a sensitivity of 56% and specificity of 92%. However, as shown in Table 10, when comparing parasite-positive cases and parasite-negative cases there is a difference in the percentage of reactivity. The biological significance of this finding, even though the sample was small, remains unknown. Positivity relationship with age was significant in high risk groups (ages 10-40 years old) and also regarding time of evolution (83% of positive cases were < 6 months).

Table 9. ELISA results for the different groups studied

Group	No. of sera	No. of positive	Percentage reactivity
I. Healthy-skin test negative	40	12	30 %
II. A) Mex.LCL, P(+), ELISA (+)	21	9	7.5 %
B) Mex.LCL, P(+), ELISA (-)	9	5	55.55%
C) Mex.LCL, P(-), ELISA (+)	9	9	88.88%

Mex.LCL= cases; P(+)= parasite-positive; ELISA (+/-)= ELISA IgG.

Table 10. Sensitivity of IgM-ELISA to detect antibodies anti-*L. mex. mex.* in patients with Mex. LCL related to parasitological demonstration

Group	No. of patients	No. of positives	Percentage of reactivity
Parasite-positive	30	14	46.66%
Parasite-negative	9	8	88.88%
Total	39	22	56%

Results and Application to Prevention and Control

Health research is the generation of new knowledge using the scientific method to identify and deal with health problems. Knowledge, worldwide or local, is essential for effective action. Local knowledge is essential to: 1) identify and set priorities; 2) guide and accelerate application of knowledge to solve health problems; 3) develop new tools and strategies; and 4) advance basic understanding (30). The present review of Phase I and II projects results supported by IDRC from 1987 to 1991 regarding leishmaniasis in the State of Campeche, Yucatan Peninsula, an endemic area recognized worldwide for Chiclero's ulcer, has generated the following benefits.

On the basis of diagnosis tool development as the first step in the control (both at the individual and community levels), it was possible to obtain reliable knowledge

regarding the magnitude of this health problem in an endemic area of Mexico. As a consequence, the national health authorities included leishmaniasis as a notifiable disease in Mexico and designated our Tropical Disease Research Unit, Research Center "Dr. Hideyo Noguchi" as a Reference Center responsible of the "Programme for the Study, Surveillance and Control of leishmaniasis at the Peninsula of Yucatan".

This program includes four main objectives: 1) To carry out multi- and interdisciplinary research including social, economical, cultural, epidemiological, clinical, parasitological, entomological, zoological, immunological and cellular-molecular biological studies; 2) To organize an epidemiological surveillance system; 3) To organize an efficient system of medical care; and 4) to promote community participation.

In order to achieve these objectives it has been necessary to improve and increase resources and staff development (training activities, post-graduate courses, etc.). It has also been necessary to establish accurate coordination with Rural Health Post personnel from both IMSS-Solidaridad Programme and Secretaria de Salud. This has included training for detection (suspicious "cases"), standardizing notification procedures and records (specific format, age, sex, place of infection, date, lesion, and skin-test), and monthly field work to confirm diagnosis (clinical history, parasitological demonstration by smear, isolation-culture, biopsy and immunological studies). It has also been necessary to carry out clinical trials to evaluate the safety and efficacy of anti-leishmania drugs; to organize and supervise Rural Health Committees and Health Promoters in each "ejido" (small village); to inform to local authorities and communities of the activities (research proposals) prior to initiation, during development, and at the conclusion of studies (conveying findings, results and benefits).

Main Conclusions

Diagnostic tools: It is well known that diagnosis tests, such as the ELISA of this project, applied to "community diagnosis" are a pre-requisite to the planning, implementation and evaluation cycle to determine the effects of the disease and the control strategies selected according cost/benefit analysis.

Epidemiological studies: Since Phase I and subsequently through the Phase II Risk Factor Study, it has been possible to determine: incidence; prevalence; distribution by time, location, age, sex, occupation etc.. It has been remarked that the most important knowledge obtained has been the identification of the "high risk population", i.e. target to which to apply any selected control strategies.

Clinical studies: To date we have studied more than 400 leishmaniasis patients. It has been possible to study clinical presentation (patterns); histopathological patterns; and evaluate the safety and efficacy of anti-leishmanial drugs.

Parasitological study: We are just at the beginning of this very important study to

characterize the focus, i.e. identify specie or species responsible of the clinical forms studied. We must point out, that we have developed and acquired staff, resources (equipment, supplies and chemical reagents) to carry out biochemical characterization of "stocks" isolated from human cases to date. Furthermore, we have also obtained the resource necessary to identify parasites isolated from vectors and reservoirs (to be initiated in 1992).

Entomological study: We have completed the one-year first study in the endemic area. Results have been briefly commented upon. We must remark that this base (control) study was done mainly to evaluate and exclude peri-domiciliary and/or intradomiciliary transmission. Data obtained have been analysed in correlation to epidemiological and clinical studies (case detection, place of infection) and reinforce the possible role of sylvatic transmission (study to be initiated in 1992).

Community participation: The research team has received stimulating acceptance from the community with respect to each proposal presented to them and developed. They have even said "we will accept any suggestion to prevent and control this disease". We most mention that we have also started social-economic studies in collaboration with the University of Calgary, Canada, which are being supported by TDR/WHO.

Immunological studies: It has been possible to develop enzyme-linked immunoassays to detect IgG and IgM antibodies to L. mex. mex. promastigotes.

Possible application to prevention and control: With all these data analysed as a total and representative sample of "reality" from the endemic area studied, it is possible now to establish "well defined" case diagnosis based on epidemiological, clinical, therapeutic response, histopathological, parasitological and immunological criterion (which are included in the Leishmaniasis Programme). Through active community participation it is now possible to detect, notify, confirm diagnosis and treat cases. It has also been possible to carry out biological (immunological, parasitological, molecular biology studies to advance in our host-parasite relationship knowledge), and follow-up on a yearly basis on the the patients who have been studied. This feature will be very important and useful for the vaccine research which is to be initiated in 1992. Due mainly to the positive and supportive attitude of the community, our primary objective is now to design realistic control measures based on community participation. Research in this area is to be initiated in 1992.

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Risk Factors and Leishmaniasis: Possible Contributions for Control Strategies¹

J. Calmet Böhme²

Introduction

The risk factors originating from or represented by "classical biomedical factors" are rather easily identified in our minds, but some risk factors and their contributions are not so obvious and further work is required for their clarification. The latter are more related with social, cultural, and economic factors. The identification of these risk factors can especially contribute to the design of appropriate control strategies.

Our work has been carried out in a province of Cuzco, whose inhabitants are mainly peasants. They live at altitudes over 3,000 m.a.s.l., where there is no primary transmission of leishmaniasis. However, due to economic problems they need to go to the nearby amazon region to work in the gold mines. It is here that they are exposed to the disease. In the national context, leishmaniasis from Cuzco has historically represented near 50% of all the disease reported in Peru (Graph 1).

As a conceptual framework, we have tried to understand disease or the lack of health not only as a biological problem of individuals. We have attempted to explore leishmaniasis in its real dimension as a health problem, and particularly how society and social conditions influence the epidemiology of the disease and vice versa.

With this analytical approach, we are trying to construct an comprehensive and diachronic exploration of the epidemiology of leishmaniasis. Although schematic, such an epidemiological model can help to summarize the complex mechanisms through which the different elements and categories (both social and biological) interact and can be used to compare each reality. A complex methodological design has been needed for this approach. Different quantitative and qualitative techniques, from distinct disciplines, are to be used.

Methods and Results

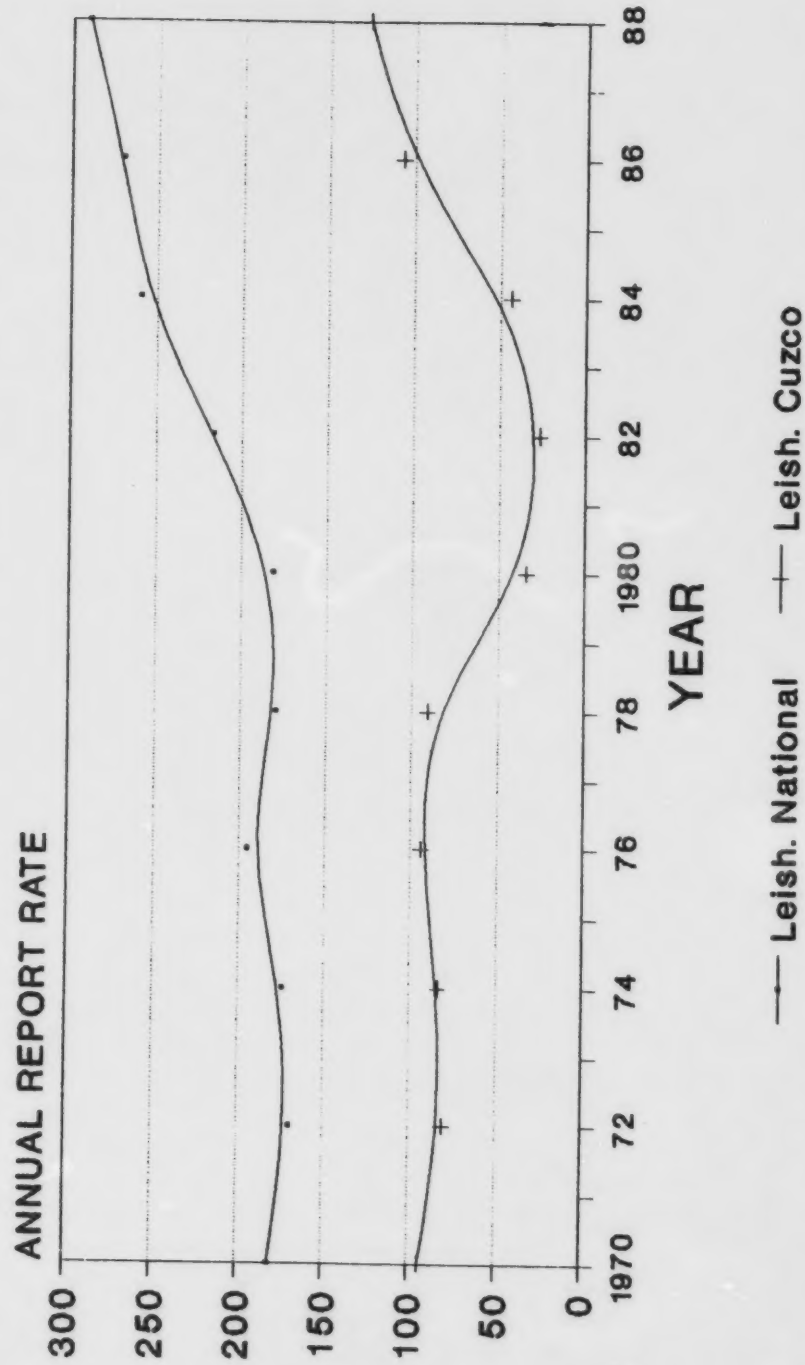
This paper presents the results of the first tool we have applied in Ocongate, a district in Cuzco. After a meeting with the community presidents or their representatives in which they agreed to participate in a community survey, a community assembly was

¹ This document was originally prepared in spanish for the workshop. This translated version is a summary of the original document thanks to the invaluable collaboration of Dr. Jaime Chang Neyra of the IMT "Avon Humboldt".

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GRAPH 1

OFFICIAL STATS. LEISHMANIASIS



scheduled for each visit. We visited 37 communities and between 50-80% of all adults in every community came to the meetings. Each adult was asked for their migratory experience and leishmaniasis history.

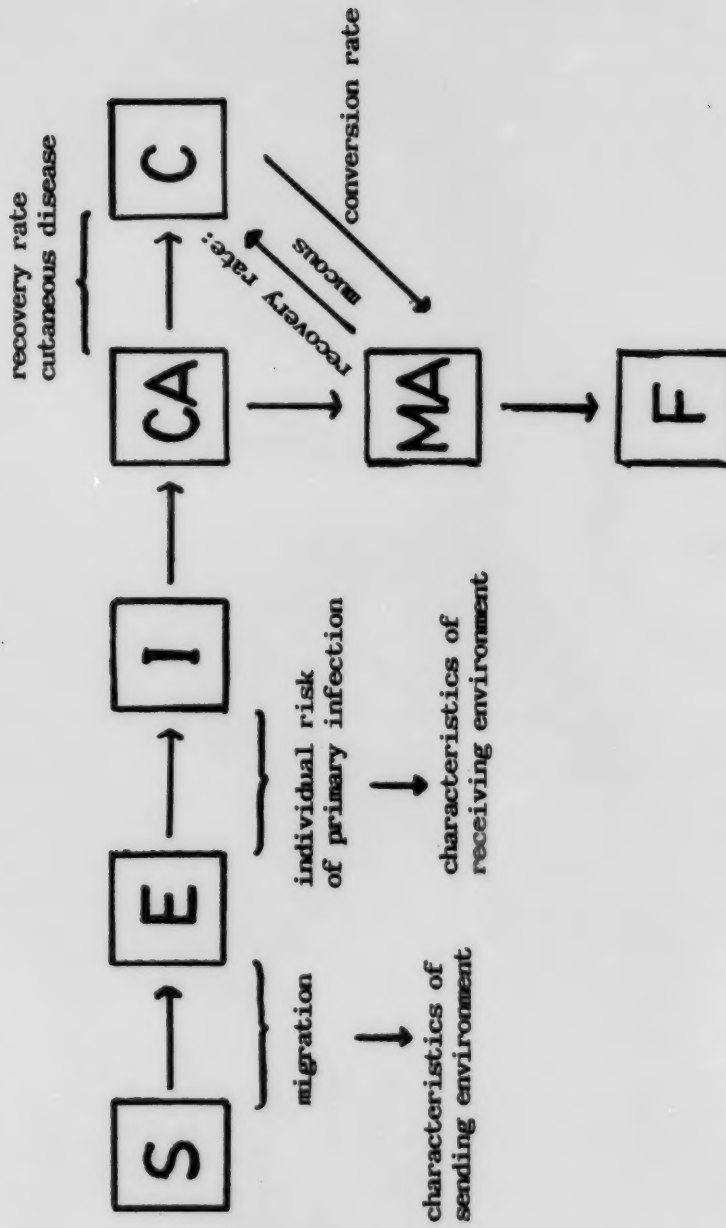
In trying to make a concrete contribution to control strategies, our work aims to define the principal elements that explain and predict the epidemiology of leishmaniasis, particularly its transmission, based on the past epidemiological behaviour of the disease in this population. It also seeks to define the structure and demand for health services currently and in the future. Our team believes that the successful control of health problems must result in a balance between the demand for health services and the response to this demand by the health services.

Scheme 1 summarizes the principal hypothesis we are using for studying the epidemiology of leishmaniasis in the Ocongate region. This model was originally developed by other Peruvian researcher, Dr. Miguel Campos, and we have adapted it to the specific context of the South Andean region. Box S represents the susceptible population; people who have never had the disease or been infected, and that reside in the highland where no primary transmission of the disease occurs. Box E represents the exposed population; the people that in accordance to a migration rate go to the jungle and get exposed to leishmaniasis transmission. The migration rate depends on the economic, social and cultural factors of the highland population which is migrating. Box I (and CA) represent the population that become infected and develop cutaneous disease when they go to the jungle. They get infected in accordance to a rate we call risk for prime infection, in last instance this depends on the specific activity undertake in the jungle. Box C represents the people that suffered the cutaneous disease and, as it usually occurs, heal spontaneously in accordance to a specified recovery rate. Finally, box MA represents the population that develops mucous disease; in most cases people who have a cutaneous scar and develop the mucous form of the disease in accordance to a specific conversion rate.

The results we are presenting are gathered in relation to these hypotheses. First, we will present the conventional indicators of leishmaniasis, the incidence and prevalence of the different clinical forms of leishmaniasis found in Ocongate. This will be followed by a discussion of the direct determinants of these conventional indicators. In the case of cutaneous disease incidence, the determinants which seem to be the most important are the migration rate to the amazon region and the risks of primary infection. In the case of mucous disease incidence, it seems to depend principally on the conversion rate.

Finally we will present some advances in the identification of the indirect determinants of the epidemiology of leishmaniasis. Particularly, some socio economic factors that trigger and influence the magnitude of the variable that in some way initiates the epidemiological chain, that is, the migration rate to the jungle. We will try to identify what are the economic, social and cultural factors which characterize the migrants to the jungle.

SCHEME 1



S : POPULATION
 E : EXPOSED POPULATION
 I : INFECTED POPULATION
 CA : POPULATION INFECTED WITH CUTANEOUS DISEASE
 C : POPULATION WHICH HEAL SPONTANEOUSLY
 MA : POPULATION WITH CONVERSION TO MUCOUS DISEASE
 F : DEATH

Conventional Indicators of the Epidemiology of Leishmaniasis

As anticipated from official statistics, leishmaniasis is an important health problem. Nearly one out of every six men in Ocongate has suffered the cutaneous form of the disease sometime during his life. This proportion increases to one out of every three in the group between the second and the fourth decades of life. Almost 80% of cases occurred in men between 15 and 35 years of age. On average, more than 5% of the people that were free from disease in 1985 were found to have the disease at the time of this survey and this proportion increases to 20% among men in the second and third decade of their lives.

The case of mucous leishmaniasis is no less important. Almost 5% of all men interviewed had mucous involvement sometime during their life and when men reach the fifth decade of their life, 10% of them have already had mucous disease.

Leishmaniasis in its different clinical forms is extremely rare among women. Less than 2% of the women surveyed had suffered cutaneous disease, and only two out of 1,300 women interviewed had mucous involvement.

Direct Determinants of Epidemiological Indicators

As we mentioned before, two very important determinants have been identified in relation to cutaneous leishmaniasis. The first is the migration rate, since there is no primary transmission in Ocongate. Second, the risk of primary infection, that is the probability of becoming infected or sick while remaining in the jungle. Both factors were explored in our survey in Ocongate in order to explain the epidemiology of cutaneous leishmaniasis.

Migration to the jungle from Ocongate is a very frequent event among the male population. On average more than 50% of the men have gone to the jungle at least once in their lives. This proportion increases to 80% among the population in the second and third decade of life. The male population of Ocongate, on average has migrated three times to the jungle and, currently, by the time a male reaches the fifth decade of life at which point their migratory life has probably ended, they have on average undertaken five migratory episodes to the jungle.

There are two prominent features in this migration. First, working in the gold mines is the principal reason for migration among all age groups, accounting for more than 80% of the episodes. Second, the temporal character of the migration is such that 75% of the episodes last three months or less, and another additional 20% last less than 12 months.

The history of more recent migrations indicates that one third of the male population has travelled to the jungle during the period 1985-1990, and that for some age

groups that proportion increases to two thirds. Many of the males interviewed referred to at least one migratory episode for the same quinquennium, which represents an annual migration rate close to 20%. For those aged 10-29 years that value increases steeply, reaching an annual migration rate of nearly 40%.

As it was mentioned before, the risk of primary infection (i.e. the chance of each susceptible person to get the infection/disease in a migratory event) is the other crucial direct determinant of cutaneous disease incidence. This seems to depend on the type of activities that the migrant undertakes in the jungle and on the nature of the exposure of the individual to the primary forest, the best ecological habitat for the reproduction of the zoonosis.

We have estimated the risk for primary infection for the population of Ocongate. However, we have not done a differential analysis for each attraction context. Graph 2 we present a global value of risk for primary infection for the total migratory experience of Ocongate peasants. As the mean survival curve for this variable demonstrates, when migrants reach an average of 10 migratory events the prevalence of cutaneous disease reaches 50%. That might mean a 5% probability of acquiring the disease for each migratory event.

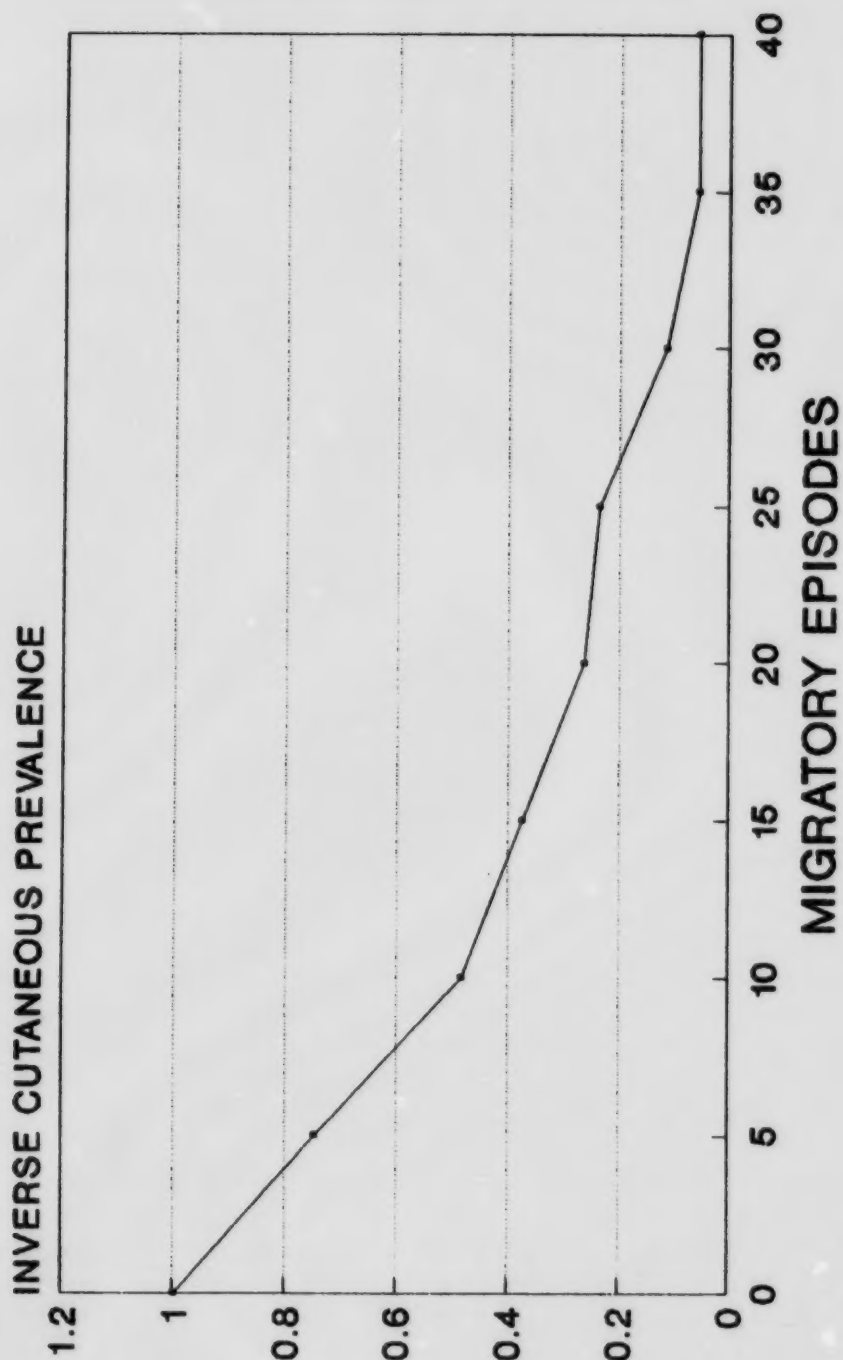
In the case of the mucous form of leishmaniasis, the crucial direct determinant seems to be the conversion rate from cutaneous to mucous disease. Typically, after one year of disease, most of cutaneous lesions heal spontaneously. Nevertheless, after healing a proportion of healed patients develop mucous disease, basically as a metastasis of the primary lesion. The annual proportion of the infected population converting is what we call mucous conversion rate; a variable that seems to depend on the biological specificity of each ethnic group involved.

The mucous conversion rate has been calculated for the sample studied by our team in Ocongate. As it can be seen in Graph 3, the mean survival curve shows that when ten years have passed from primary infection, the accumulated prevalence of mucous leishmaniasis reaches 20%. That means that with a short confidence interval, the annual mucous conversion rate is a little more than 2%.

Interesting associations can be made relating the direct determinants with the epidemiological indicators of disease found in Ocongate. Additionally, the values found permit us to predict what are the expectations for prevalence and incidence of the different clinical forms of leishmaniasis. The most important relationships found can be seen between migration rates and the epidemiological indicators of leishmaniasis disease. In summary the age groups that have the higher values for migrants and migratory episodes over the last quinquennium are the same as those which have the higher incidence of cutaneous leishmaniasis. Therefore a strong association between both phenomena is indicated.

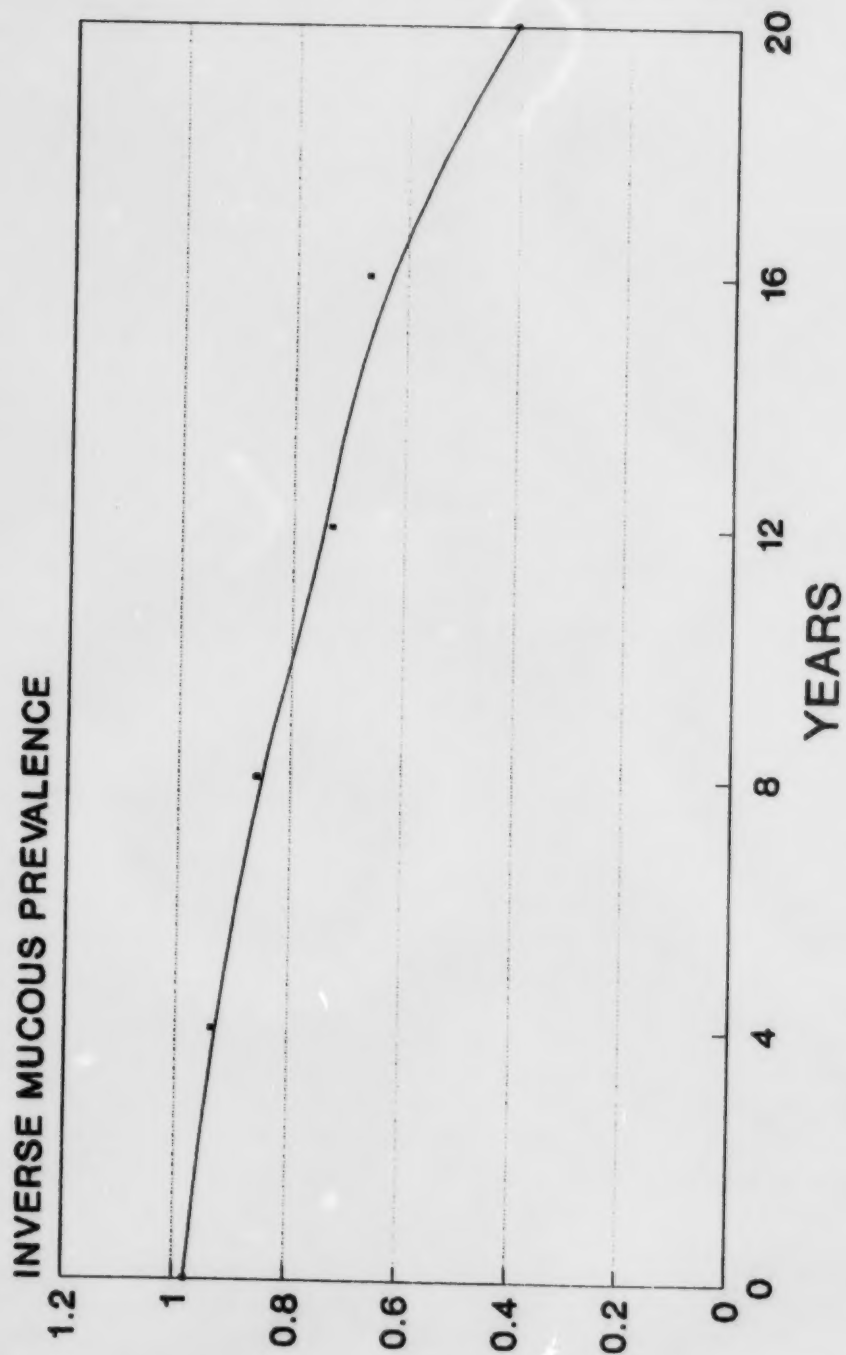
GRAPH 2

TOTAL RIPLEISH



GRAPH 3

MUCOUS CONVERSION RATE



Nevertheless, the distribution by age of the accumulated prevalence of cutaneous disease calls attention. It would be logical to expect that most of the migrants to the jungle begin their migratory experience while young, i.e. with enough physical strength to tackle the hard labour conditions of gold mining. But, if young people are the most important age group that acquires the infection or disease, some explanation is needed for the fact that older age groups have a lower accumulated prevalence of leishmaniasis, a condition that by definition is not reversible.

We think that changes in the migration rate can take account for this observation. The 20% migration rate that we see today is the final result of a dynamic process over time. Men that today are aged 40 years and older were exposed to lower migration rates and therefore prevalence of disease among this group is lower. Because migration rates have been increasing over time, the younger generations have been exposed to greater migration and as a consequence have a higher accumulated prevalence of cutaneous disease.

Acceleration of the migration rate seems then to be the most important risk factor that by itself can modify the accumulated prevalence of cutaneous disease and also the accumulated prevalence of mucous disease, which depends on the former.

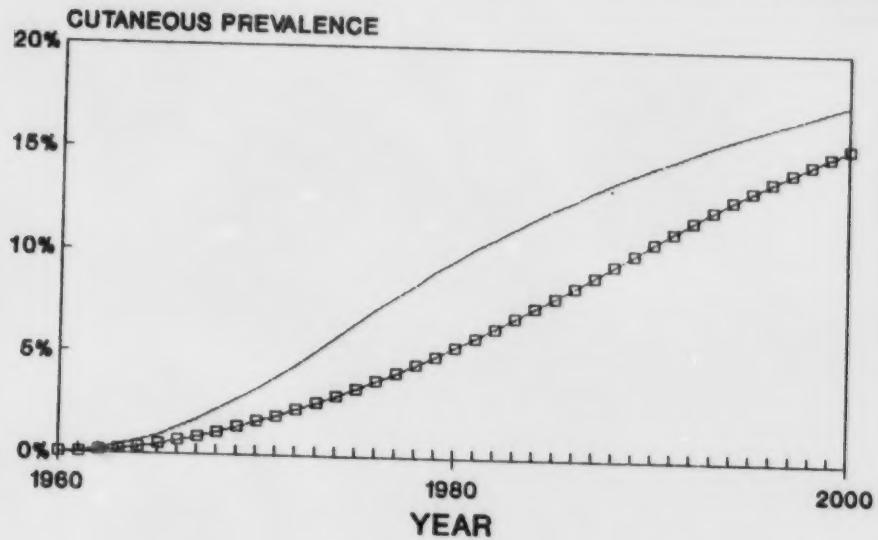
Accordingly, it should be expected that a higher acceleration of the migration rate will condition the occurrence (within a certain period afterwards) of higher incidences of the different forms of disease and higher accumulated prevalences, thus increasing and concentrating the impact of disease on people and the health system.

We have tried to demonstrate this effect with a mathematical simulation done in a personal computer. Stable values have been used for the principal direct determinants of both clinical forms: the maximum migration rate, the risk of primary infection, and the mucous conversion rate. We introduce different accelerations, one the double of the other. When higher accelerations of migration rates are used the slope of the curve changes, shifting left and upwards (Graphs 4 and 5).

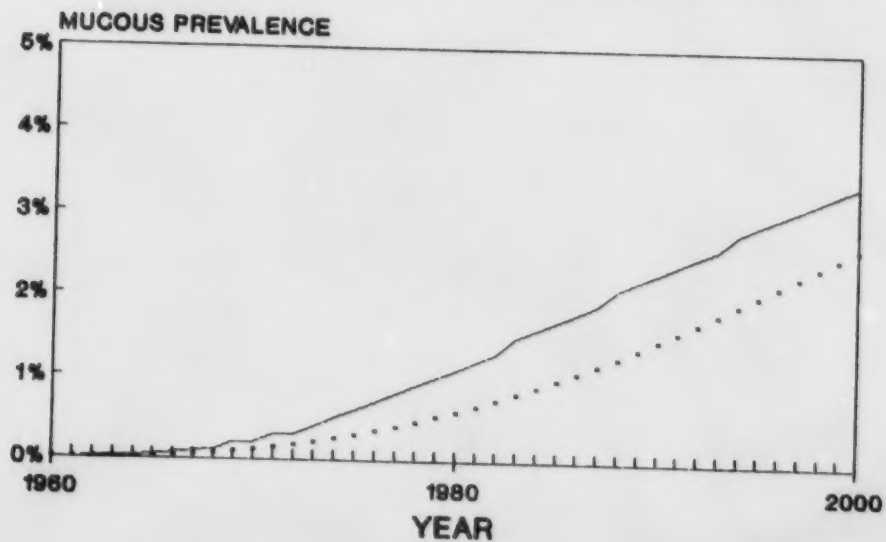
Graphs 6 and 7 show the additional proportion of cases of leishmaniasis observed with changes in the acceleration of migration rates. When the population reaches a maximum annual migration rate of 20% in 15 years instead of in 30, the proportion of additional cases (and then the increase of disease impact and its recuperative costs) grows between 20% and 100% for cutaneous leishmaniasis and between 40% and 100% for the mucous form of the disease.

Although the principal direct determinants could remain stable in the future, including the acceleration of migration rates, the forecast for the future epidemiological situation of the disease is extremely worrying. The average number of migratory events for the male population approaching the fifth decade of life, is rapidly reaching a value which corresponds with a 50% rate of prevalence of cutaneous lesion on the mean

GRAPH 4 CUTANEOUS PREV. AND ACCELERATION

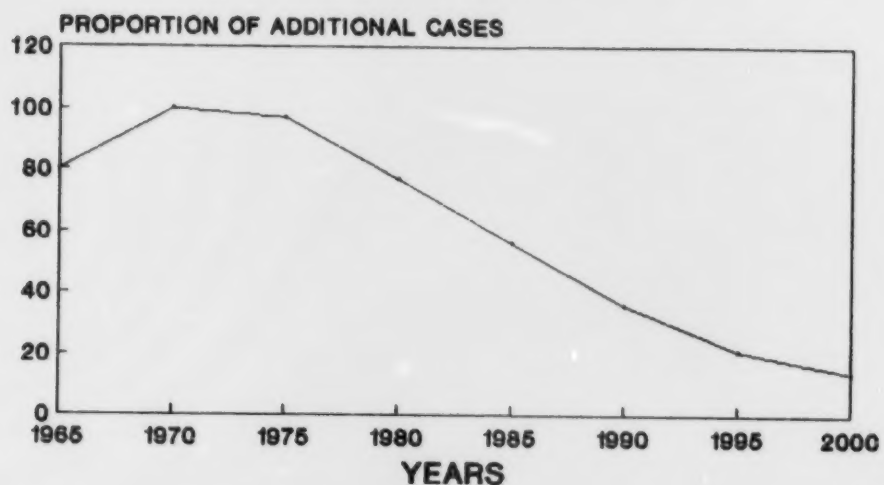


GRAPH 5 MUCOUS PREV. AND ACCELERATION



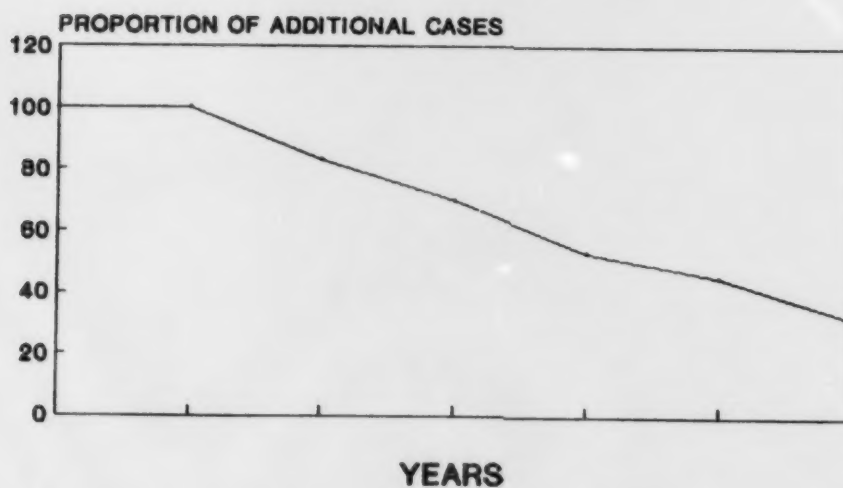
GRAPH 6

EFFECT OF ACCELERATION CUTANEOUS CASES



GRAPH 7

EFFECT OF ACCELERATION MUCOUS CASES



survival curve. As a result we expect that in a short time the prevalence of cutaneous disease for this age group will be 50%, meaning that one out every two peasant families will have to assume the health impact and costs of disease within their households.

The situation for the mucous form of leishmaniasis, which has a enormous social impact and recuperative costs, has a similarly bad prognosis. Almost 10% of the male population will suffer from mucous leishmaniasis by the time they reach the fourth decade of life. This means that one of every 10 peasants families will have to assume the high impact and costs of mucous leishmaniasis in their households.

Indirect Determinants of Epidemiological Indicators

Migration as a social process, is the result of the cross action effect of a variety of economic, social and cultural factors that in a generic way we called indirect determinants of epidemiological indicators of the disease. It is principally the historical context of South Andean societies that explains the appearance of the migratory phenomenon, and its modification over time.

In the most strict economic sense, for the migration of the Andean peasant population to the Amazon expresses the need for individuals to sell their manual labour because of the unequal terms of their access to the market economy. It is ultimately the final result of a broad range of unequal exchange relationships that denies the peasant society the benefits of urban centers, firstly of the cities in the region, then in the capital of the Republic, and finally, in the profit of the industrialized northern hemisphere.

The peasant migrates because he or she needs the salary to subsist and sustain his family. The migrant peasant is pushed to sell scarce crops at very low prices and to buy expensive manufactured goods which are needed. The generated deficit must be covered with the only asset the peasant still retains, their manual labour.

Some of the results of our research reinforce our hypothesis. The variation in migration rates that can be observed in Ocongate can be explained by the increasing deterioration in the terms of exchange for the peasant society of the region. A group of the peasant communities located in the areas of higher altitude and especially dedicated to cattle, are migrating more recently with higher rates because of the abrupt decrease in the international prices of their principal cash product: alpaca wool.

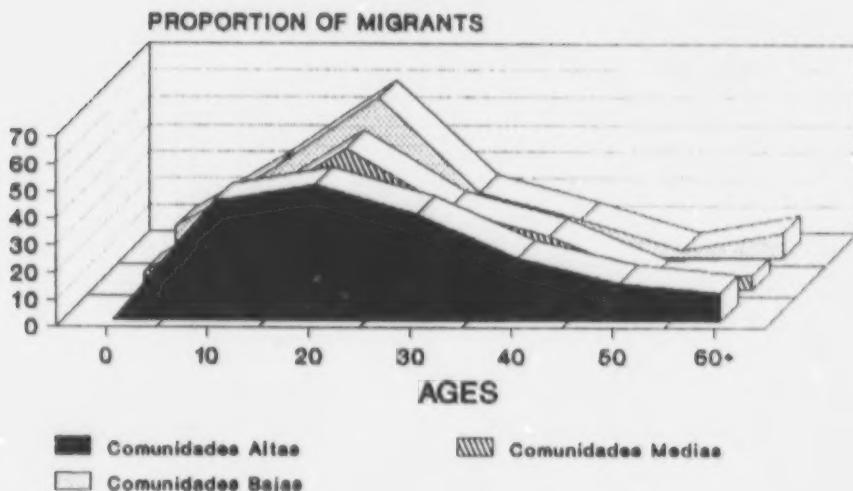
To understand the difference in migration rates and the incidence of cutaneous leishmaniasis in Ocongate we divided the community in three ecological and socio-economic habitats. Each one has some unique characteristics as shown in Table 1. The characteristics range between two extremes. On one side there are the communities of the higher elevations, which occupy the "puna" ecological levels over 4,000 m.a.s.l., dedicated to cattle, with "traditional" socio-cultural patterns, with "scattered settlement" patterns, and recent articulation with the official health service. On the other

TABLE 1: Ecological, Economical, Socio-Cultural and Sanitary Differences in Ocongate District

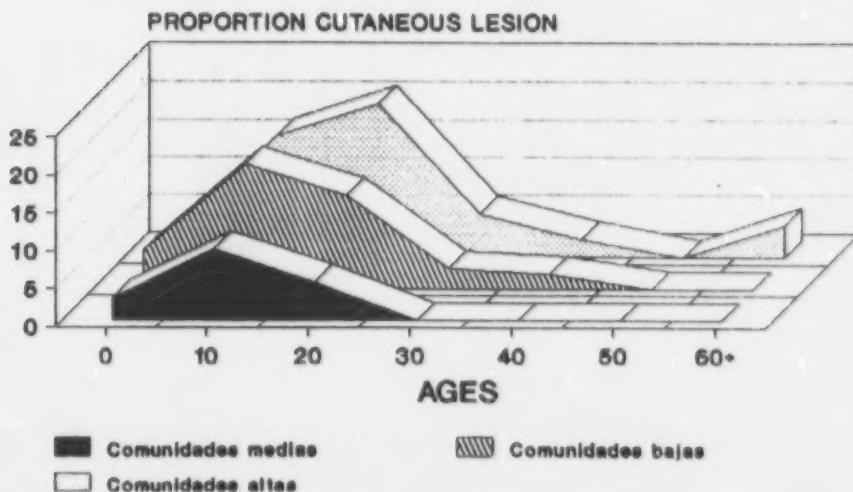
	Predominant Ecological Pattern	Predominant Conomical Pattern	Predominant Socio-Cultural Pattern	Predominant Settling Pattern	Predominant Sanitary Pattern
H I G H C O M M U N I T Y	Ecological floor: - Suni alto/puna Altitude: 4,000-4,800 m.s.n.m.	Principal Productive Activity: Cattle: Auguenids (Alpaca)	- In general, more "traditional" - Emphasis in production for "self-consumption" - hand labor: relationships of reciprocity and mutual aid	- Of scattered type, rural	- Recent articulation with official health services
M E D I U M C O M M U N I T I E S	Ecological Floor: - Suni bajo Altitude: 3,700-4,000 m.s.n.m.	Principal Productive Activity Combine: - Cattle (auquenid, sheep) - Agriculture (potatoc)	- Intermediate pattern	- Combine type, semiurban and rural	- Intermediate pattern
L O W C O M M U N I T I E S	Ecological Floor: - Quechua Altitude: 3,300-3,700 m.s.n.m.	Principal Productive Activity: Agriculture: - Maize - Barley	- In general more "modern" - Little emphasis in production for "self-consumption" - Hand labor: relationships of "asalarated" type	- Concentrated type, semiurban	- Old articulation with official health services

SUMMARY OF GRAPHS No. 9

JUNGLE MIGRANTS 85-90



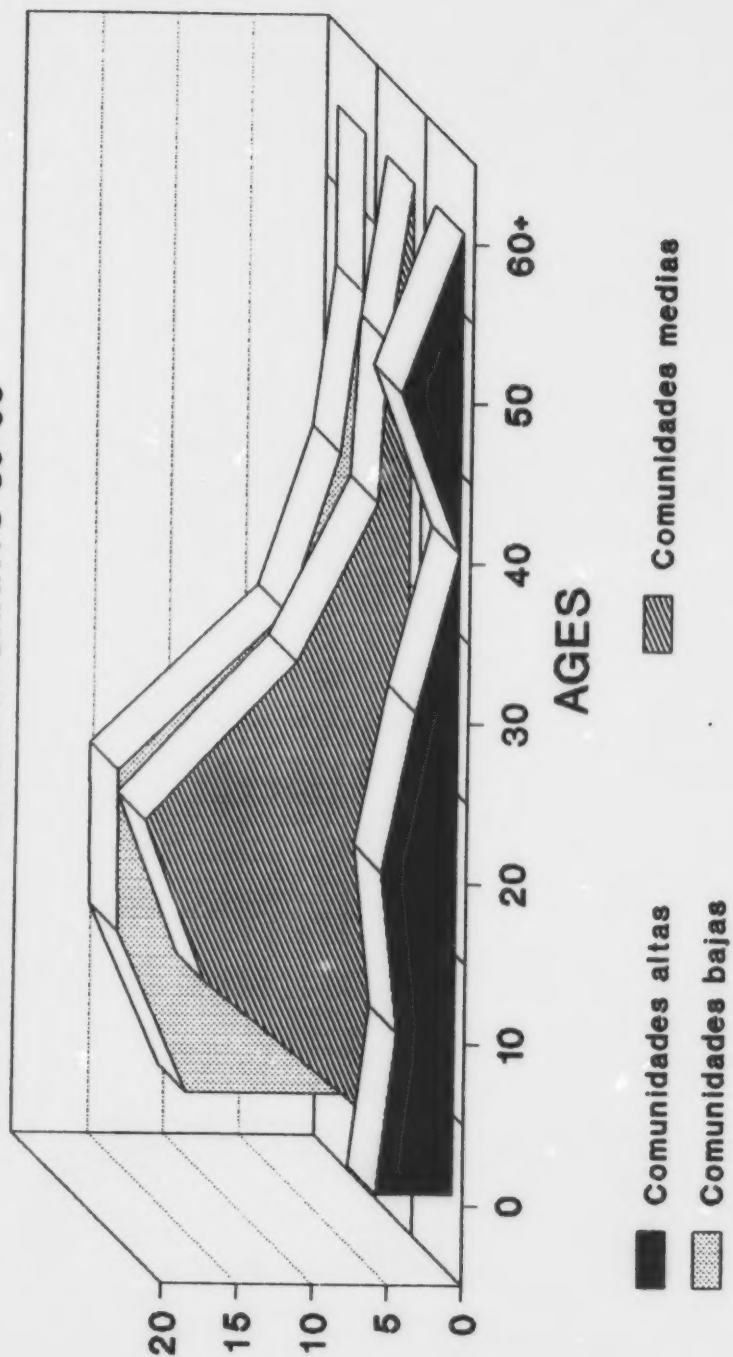
CUTANEOUS INCIDENCE



GRAPH 10

WOMEN MIGRANTS 85-90

PROPORTION WOMEN MIGRANTS 85-90



extreme, there are the communities of lower elevations, which occupy the "suni" ecological levels, above 3,000 m.a.s.l., dedicated to agriculture (especially to maize and potato production), with "modern" social-cultural patterns, concentrated or semi-urban settlement patterns, and an established articulation with official health services.

In the summary Graphs 9 we first present the age distributions of the recent migratory experience. Although, on average the three groups of communities exhibit the same values for recent migrants, it can be early noticed that in the case of the communities of high elevation the average age of migrants seems to be lower. That is, age groups that because of their youth could only migrate in the last period. From this we can say, that the configuration of the curves of recent migration suggest that the communities of higher elevation will be migrating more intensely than the other groups of communities. The fact seems to be confirmed in the last graph of this summary where we can clearly see the higher incidence of cutaneous disease among communities of higher elevations, especially among their younger inhabitants.

The similarities in the recent migration experience (curves) and incidence of disease for the different groups of communities, seems to be related to economic changes that occurred recently. Usually the group of communities which had greater migration rates were the ones from the lower elevation due to the low prices of their cash crops. In the past, the good price of alpaca wool used to protect communities of the higher elevations from the need to migrate. However, the abrupt fall of alpaca wool prices has recently pushed the latter community group to migrate to the jungle. We expect that this change will cause a rapid increase of the incidence and prevalence of leishmaniasis, creating a need for specific control measures.

Graph 10 shows the distribution by age of female migrants in the three groups of communities. The proportion of female migrants is considerably higher for the low and medium altitude communities. It appears that some kind of limit to male migration is being reached in some communities. Since these communities cannot expel more male population to the jungle and still sustain vital agricultural activities in the highlands, they instead expel women and youngsters, even children, to get the income their families need.

Conclusions

Some preliminary conclusions can be made from the results presented regarding to control strategies for leishmaniasis in the South Andean region of Peru.

1. In the first place, leishmaniasis has been demonstrated to be a major public health problem in the region, especially among the male population of economically active age. In the short term, about one out of every two households will have suffered the impact of cutaneous disease and one out of every ten households will have suffered the effect of mucocutaneous disease.

Specific control strategies for solving the problem should be developed promptly, because both the social impact and the recuperative cost of the disease are cumulative.

2. Temporal migration for gold mining is the direct determinant most closely related to incidence of the disease. The magnitude of this migratory phenomena is huge, reaching annual migration rates of 20% in Ocongate with the migrants being predominantly young males. Any sustained positive modification of leishmaniasis incidence will depend upon improving social and economical opportunities, which require specific political decisions. Changes in migration rates occur differently for different communities, and as a consequence the occurrence of leishmaniasis is greater in some communities in which both the impact of disease and its recuperative costs tend to concentrate over time.
3. Sanitary education should be a vital part of any control strategy in order to develop a collective awareness of the problem, and also to create a collective willingness to change the conditions that make the problem occur, including those factors which are social and economic. For this, an active and horizontal exchange of knowledge and experiences between the different social actors, community members and leaders, health and social scientists, authorities, etc. needs to be stimulated.
4. It is urgent to establish control interventions which diminish the expected incidence of disease with high efficiency and efficacy if the overall impact of leishmaniasis is to be reduced, taking into account social and economic factors.

Three Research Perspectives on Transmission Related Risk Factors for Cutaneous Leishmaniasis in Costa Rica

I. New Strategy for the Control of Cutaneous Leishmaniasis: The Case of Acosta, Costa Rica

J.C. Rojas¹

Introduction

In a previous study in Acosta on cutaneous leishmaniasis, we estimated the odds ratio (OR) for several variables (1). Due to a previous lack of knowledge in intra and peridomestic risk factors, that pilot study shed light onto some of the variables that could be associated with the transmission of the disease. At the same time, it initiated a larger study which is presently being carried out. Among the objectives of this new study are: 1) to validate the findings of the pilot study; 2) to explore new potential risk factors for disease transmission; and 3) to acquire the knowledge to develop a specific strategy for the control of the transmission of cutaneous leishmaniasis.

For such control several measures have been taken around the world. These vary according to the ecological characteristics of the disease (vectors, reservoirs and the environment where transmission takes place). Examples of these control measures include: leishmanization, vaccination, deforestation, use of residual insecticides, host protection, etc. Some of these have been successful only in certain areas, others have been only partially effective and others have not been effective at all. In none of these experiences was the impact of the measure or intervention, estimated before it was implemented. This means that there was no previous knowledge of their impact in reducing the incidence of the disease.

The population attributable risk percent (PAR%) estimates the proportion of disease that is reduced in the population when a variable or factor is controlled. In other words, it is the proportion of cases in the target population attributable to exposure to a given variable.

In a multifactorial disease such as cutaneous leishmaniasis, factors related to the host, the vector, the reservoirs and the environment in general, play an important role in the transmission dynamics of the disease. Therefore, it is very important that the investigator and especially the health authorities know which factors are involved in

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transmission and what proportion each of them contributes. This is basic knowledge that should be known before designing control measures. Once we have this information (PAR%), it is necessary to consider the economical and practical feasibility of implementing the measure. At the same time, we also have to consider the acceptance of the intervention by the community in question; is the measure consistent with their culture, beliefs and values? These are questions to be asked before initiating any intervention.

In this preliminary report, I will show the use of the PAR% as a tool for planning control in a directed and intelligent approach.

Materials and Methods

The population under study is the county of Acosta, the characteristics of which have been described previously (1). The data comes from an on-going study that started in 1989 and is planned to end in 1992. The sample size used in this interim analysis consists of 105 houses, with 29 cases and 76 controls.

The study design is a population case-control, with the house as the unit of interest. To be considered a house-case, a house should have a child with a recent lesion (< 6 months) of cutaneous leishmaniasis diagnosed by our personnel. This child should be < 10 years of age and with a positive Montenegro skin test. To be considered a house-control, a house should have a child in the same age range (± 2 years) without a history of disease and with a negative Montenegro skin test. Also the house-control should be within a radius of ± 100 meters from the house-case.

We are working only with incident cases; this has several advantages related to control of biases, that we would not have if working with prevalent cases. Other characteristics of the design and advantages have been previously described elsewhere (2).

The odds ratio (OR) was first estimated by constructing 2x2 tables, then a logistic regression model was constructed to simultaneously control for potential confounders. From the estimated beta coefficient of the logistic model, the OR and its 95% confidence interval (95% CI) and the PAR% were calculated.

In order to estimate the population attributable risk percent or etiological fraction we need to know: a) the prevalence of the variable of interest in the population under study; b) the estimated OR in a case-control study or the relative risk (RR) in a prospective cohort study, for the variables of interest.

The formula for its calculation is:

$$\text{PAR\%} = \frac{\text{Pe (OR-1)}}{\text{Pe (OR-1)} + 1} \times 100$$

Where : Pe is the proportion of the population exposed to risk.

OR is the odds ratio (OR) estimated for that factor.

Results

After the interim analysis of 29 house-cases and 76 house-controls, several variables were found to be associated with the intra and/or peridomiciliary transmission of cutaneous leishmaniasis in the area of study. Only 6 variables were included in the model based on previous knowledge of: 1) place where domestic garbage is discarded; 2) animals that sleep underneath the house; 3) pigs around the house; 4) high number of members per house; 5) use of latrine; and 6) presence of hen-houses.

Table 1 shows the OR's with their 95% CI and its PAR% for the variables. Houses where garbage was not discarded in an appropriate hole were 2.6 times (95% CI=1.2-4.1) more likely to be case-houses than houses where garbage was discarded adequately. The PAR% of this variable was 57%, the highest obtained, meaning that if the population under study discarded the garbage in a appropriate hole, the incidence of the disease would be reduced by 57%. The houses that were built on stilts and had domestic animals that slept at night in the space between the floor of the house and the dirt, were 3.6 times (95% CI=2.1-5.0) more likely to be case-houses than houses that did not have animals in this space. The PAR% for this variable was 50%, meaning that if the animals were removed from this space, the incidence of disease would be reduced by the 50%.

The variables, presence of pigs around the house during the day and high number of members per house (>5), presented an OR of 2.9 and 2.2, respectively. Both variables presented a PAR% of 49%. Those houses that had a latrine were 2.0 times (95% CI= 0.6-3.5) more likely to be case-houses than those that did not have a latrine; its PAR% was 46%. The last variable, hen-houses, had an OR of 0.7, meaning that it had a protective effect. This means that the houses that had hen-houses around had a 30% reduction in the incidence rate of disease compared with the houses that did not. Also, this variable had a wide confidence interval that included the null value, (95% CI=0.3-2.4) with a PAR% of 19%.

Discussion

In this interim analysis, variables that were not in the pilot study were included. Among these were: garbage disposal, animals that sleep under the house at night, pigs around the house during the day, and location of the latrine.

In the pilot study the variables, number of members (OR=6.6 95% CI=1.1-70.3), houses with stilts (OR=3.0 95% CI=0.6-13.6), animals inside the house (OR=8 95% CI=1.2-53.4) and hen-houses (OR=0.2 95% CI=0.01-0.9) were found to be associated with the the house being a case. In this interim analysis, those variables were also found associated with transmission of cutaneous leishmaniasis. This analysis considers only animals that sleep at night in the space of the stilts but not houses with stilts alone or domestic animals inside the house.

Odds Ratios (OR) in Multivariate Logistic Regression and Population Attributable Risk Percent (PAR%) for Variables Associated with the Transmission of Cutaneous Leishmaniasis in Acosta, San José, Costa Rica, 1991

VARIABLE	OR (95%CI)	% EXPOSED INDIVIDUALS POPULATION IN STUDY	PAR %
GARBAGE DISPOSAL			
OTHER VS HOLE	2.6 (1.2-4.1)	84	57
ANIMALS UNDER HOUSE			
YES VS NO	3.6 (2.1-5.0)	39	50
PIGS AROUND HOUSE			
YES VS NO	2.9 (1.9-4.0)	50	49
MEMBERS PER HOUSE			
HIGH VS LOW	2.2 (1.0-3.4)	79	49
LATRINE			
YES VS NO	2.0 (0.6-3.5)	85	46
HEN-HOUSES			
YES VS NO	0.7 (0.3-2.4)	58	19

We have to consider that in the pilot study, the analysis was bivariate, controlling only by number of members, that were found to be confounder. In this analysis the logistic procedure was used to control for several confounders simultaneously. This means that we were able to control for the effects of the other variables, so that we were able to obtain the effect of only one variable at the time.

Among the new variables, we observed that the manner of garbage disposal was associated with a house being a case. Those houses that discarded the garbage in places other than a hole presented a risk of having a child with the disease 2.6 times greater than those where the garbage was discarded in a hole. Garbage disposal had been reported as associated with the transmission of leishmaniasis (3), but had not been quantified.

Domestic animals that sleep under the house at night and pigs around the house during the day, could act as bait for sandflies, increasing the risk for children having contact with sandflies. In the pilot study we reported only the presence of this space as a risk factor. Here we are adding some information to the variable, and for further analysis we will determine if there is a particular animal species that is more important.

A high number of members per house is associated with the status of a house-case. We have reported that this variable could act only by increasing the contact rate between the host and the vector, so that the more members per house the more likely one of them would become a case (1).

The effect of the variable, use of latrine, is related to the fact that this place is a preferred resting site for sandflies (4); hence, persons using such a facility are more exposed to contact with the vector.

Hen-houses presented an adjusted OR of 0.7, (95% CI=0.3-2.4). As we discussed in relation to the pilot study, this variable was found to be protective against the transmission of cutaneous leishmaniasis. Even though in this analysis its 95% CI includes the null value, the fact that in the pilot study it was significant (OR=0.2, 95% CI=0.01-0.9) makes this variable a good candidate for intervention, with a PAR% of 19%. It is important to mention that these estimates could change once more variables are added to the logistic model. This is why this analysis should be considered as preliminary.

The prevalence of exposure variables among the population in the study directly affects the estimation of the PAR%. For example, the variable garbage disposal had a smaller OR (OR=2.6) than animals that sleep at night under the house (OR=3.6), but 84% of the houses in the population under study do not discard garbage into a hole. Therefore, when trying to implement a control measure, we have to consider not only the OR or relative risk, but also the prevalence of the exposure in the population under study. To do this the PAR% is calculated, even though the advantage of using the PAR% is seldom recognized in public health. Recently the WHO has been making an effort to implement the use of this measure in studies on malaria.

Because a logistic model assumes a multiplicative relation between the variables included in the regression equation, adjusted PAR% for individual factors will not add to the overall PAR% for all the factors considered jointly (5). The sum of the adjusted PAR% for individual factors may exceed 100% and as a result, the risk estimates may be difficult to interpret.

Recently new techniques have been developed to overcome this problem (5,6) and will be used in our subsequent analysis. Nevertheless, this interim analysis illustrates how calculation of the PAR% can be of great help when implementing control measures.

When researchers and public health officials know the PAR% of exposures for the disease of interest, in this case leishmaniasis, the strategy for controlling the disease can be implemented with a known probability of success, i.e., what percent the incidence of disease will be reduced.

However, public health officials should also know what will be the acceptance of the control measures by the community, as well as their economical feasibility and sustainability. That is why the participation of the community is essential in all stages of the investigation, so that when control measures are to be implemented, the community already knows why and we will also know if they will be accepted. In this area the participation of the social sciences is very important. Ideally we have to stratify the potential control measures based on the PAR%, acceptance by the community, economic feasibility and sustainability.

In a multifactorial disease such as leishmaniasis the identification and quantification of its risk factors is essential for integrated control. That is why research on leishmaniasis should include all the components of the transmission chain: host, vector, reservoir and environment. Even more important is the quality of the data; if this is poor, the conclusions will be poor and results will not be replicable.

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II. Phlebotominae Sandflies (Diptera: Psychodidae: phlebotominae) Associated with Human Houses in an Endemic Area for Cutaneous Leishmaniasis in Costa Rica

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Introduction

The first step to plan and execute vector control of any disease is the proper identification of target species (Metcalfe, 1982); however, in tropical America this is not an easy task in most endemic areas due to high diversity of phlebotominae sandflies and to the difficulties involved in parasite detection.

In Costa Rica, cutaneous leishmaniasis caused by Leishmania panamensis has been studied since 1946 (Peña et al. 1946). It had been possible to isolate the parasite from Lutzomyia ylephiletor and Lutzomyia trapidoi (Zeledón and Alfaro 1973; Zeledón et al. 1977); however, there had been found 18 species able to be attracted to and feed on the human host which could be involved in the transmission cycle of leishmaniasis (Zeledón et al. 1985).

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There had been collected, inside human houses where there were infected patients, the following phlebotominae species in Ceiba Este (Acosta, San José, Costa Rica) an endemic area for cutaneous leishmaniasis: Lutzomyia youngi, Lutzomyia ylephiletor, Lutzomyia shannoni, Lutzomyia sanguinaria, Lutzomyia cruciata, Lutzomyia gomezi, Lutzomyia trapidoi, Lutzomyia panamensis, Lutzomyia geniculata, Lutzomyia gorbitzi and Lutzomyia sordelli (Murillo y Zeledón 1985). Based on this type of information we hypothesized that in Costa Rica cutaneous leishmaniasis transmission could be domiciliary (Zeledón et al. 1982) and some house characteristics have been found associated with transmission of disease (Rojas et al. 1988; Rojas, 1991) then further studies are needed to associate particular species with the human housing system in endemic areas.

The objective of this paper is to report on phlebotominae species associated to human houses in an endemic area for cutaneous leishmaniasis and to provide entomological information for a broader epidemiological study of risk factors associated with disease transmission.

Materials and Methods

Description of the study areas: This study is being conducted in Acosta (county # 12, San José, Costa Rica) which is an agricultural area sited in the folds of Bustamante mountainous chain and planted with coffee, citric and ornamental plants.

Eight localities where cutaneous leishmaniasis is endemic were selected to conduct entomological monthly surveys, they were:

1. Breñón (Code: 525; altitude: 740 m; latitude: 9° 44' N; longitude: 84° 16' W)
2. Agua Blanca (Code: 114; altitude: 900 m; latitude: 9° 49' N; longitude: 84° 11' W)
3. Linda Vista (Code: 416, altitude: 927 m; latitude: 9° 45' N; longitude: 84° 13' W)
4. Hondonada (Code: 212; altitude: 980 m; latitude: 9° 48' N; longitude: 84° 13' W)
5. Chirracá (Code: 103; altitude: 1000 m; latitude 9° 49' N; longitude: 84° 10' W)
6. Ceiba Este (Code: 404; altitude: 1060 m; latitude: 9° 45' N; 84° 10' W)
7. Sabanas (Code: 525; altitude: 1100 m; latitude: 9° 44' N; longitude: 84° 16' W)
8. Ococa (Code: 202; altitude: 1100 m; 9° 48' N; longitude: 84° 12' W)

In this study the human house has been identified as our sampling unit and each house has been divided in domiciliary and peridomiciliary areas; peridomiciliary area usually includes animal installations, latrines and a small garden with vegetation.

Sampling methods: Selection of houses (location, number and type) within each locality was made based on epidemiological criteria previously discussed (Rojas and Zeledón, 1990). The entomological survey in each house was organized considering the following:

- a) period: diurnal or nocturnal.
- b) type: active search in resting areas during diurnal hours and passive using CDC light traps during nocturnal hours.
- c) frequency: once a month (one day) for each locality during the study period (since January, 1990 to September, 1991).
- d) traps location: one inside each house and one outside in peridomiliary areas; traps were located 1 m height.
- f) number of traps: 2 per house, however we put only one where people do not allow us to locate one inside.

Sand flies collected were properly labeled and transported to the laboratory.

Laboratory analysis: In the laboratory, all specimens were counted, sexed and identified based on genital and cibarial structures using as a guide the papers by Murillo and Zeledón, 1985 (Murillo and Zeledón, 1985) and the original description of each species referenced in the above paper.

Each species has been identified with a code as follow:

(03) Warileya hertigi; (04) Warileya rotundipennis; (05) Lutzomyia cruciata; (06) Lutzomyia gomezi; (08) Lutzomyia longipalpis; (10) Lutzomyia rosabali; (15) Lutzomyia reburra; (18) Lutzomyia ylephiletor; (22) Lutzomyia carrerae thula; (27) Lutzomyia odax; (29) Lutzomyia youngi; (30) Lutzomyia serrana; (31) Lutzomyia ovallesi; (34) Lutzomyia vespertilionis; (35) Lutzomyia viriosa; (38) Lutzomyia zeledoni; (41) Lutzomyia shannoni; (45) Lutzomyia hartmanni; (46) Lutzomyia sanguinaria; (49) Lutzomyia barrettoii majuscula; (51) Lutzomyia runoides; (52) Lutzomyia atroclavata; (56) Lutzomyia pia; (57) Lutzomyia trinidadensis; (59) Lutzomyia gorbitzi and (62) Lutzomyia bispinosa. The occurrence of Lu. bispinosa in Costa Rica has been recently reported (Herrero and Jiménez 1991).

Females were divided in three groups:

1. engorged females with fresh blood were stored at -20 °C for future blood meal source identification.
2. a representative part of each sample were dissected and cultured to isolate the etiological agent.
3. the rest of the material collected was mounted in Hoyer medium and maintained as a reference collection in the Entomology laboratory.

The condition of the female regarding gravid or empty; with or without blood in the abdomen and the blood status (fresh or not) was recorded by direct observation using an stereoscope. Most of the data collected will not be presented in this paper and will be the objective of future communications.

Results

Phlebotominae abundance: Table 1 shows a summary of overall collection data obtained sampling from houses in the localities under study; tree buttresses were, also, sampled but these data are not used for this analysis.

As shown in Table 1 a greater number of sandflies were captured during nocturnal hours than in diurnal hours what is expected due to phlebotomine habits and is explained by differences on collection methods. The relationship between female abundance, inside and outside, is different among the eight communities being the number of sandflies collected inside houses greater than those collected outside in (202) Ocoa (88/54), (404) Ceiba Este (88/40) and (416) Linda Vista (165/52). It is, also, obvious that in localities (103) Chirracá and (114) Agua Blanca a smaller number of phlebotomine sandflies was collected which may be related to house structure or to the fact that they are more urban areas.

Geographical and spatial distribution: Two aspects of spatial distribution of phlebotomines are considered 1. geographical (among localities within the region) and 2. habitational (assuming the house as the habitat unit, it is the distribution within the house). In this part of the paper we assume that geographical coincidence between a disease and its vector could be used as a clue to indicate potential vectors; we also indicate that these potential vectors may be found inside human houses as well as outside.

Table 2 shows the species collected inside human houses; it is noted that most of them have been previously reported as anthropophilic in Costa Rica (Zeledón et al. 1985).

It is clear that all the species collected have been found at least once inside human houses; it is shown in Table 2 that Lu. cruciata (O = 7/8), Lu. gomezi (O = 7/8), Lu. ylephiletor (O = 7/8) and Lu. serrana (O = 8/8) are the most commonly distributed species found inside houses. Lu. shannoni (O = 6/8); Lu. youngi (O = 5/8) and Lu. pia (O = 5/8) were not present inside houses in all localities but they are among the most commonly found species outside.

TABLE 1: Abundance of phlebotominae sandflies collected in human houses by locality, period and collection site

Locality	n* Period ⁽¹⁾		Indoors		Outdoors		Overall	
			M	F	M	F	M	F
103	6	D	0	0	0	0	0	0
		N	1	4	27	74	28	78
114	3	D	0	0	1	1	1	1
		N	4	2	28	23	32	25
202	3	D	0	0	0	3	0	3
		N	65	88	24	54	89	142
212	3	D	0	0	50	9	50	9
		N	43	80	76	194	119	274
404	3	D	1	1	0	1	1	2
		N	31	88	27	40	58	128
416	4	D	2	7	68	65	70	72
		N	275	165	68	52	243	217
513	3	D	0	0	11	7	11	7
		N	16	28	21	36	37	64
525	3	D	0	0	3	9	3	9
		N	58	39	33	85	91	124
Total (n):							933	1155

* n: refers to the number of houses per locality; ⁽¹⁾ period: D diurnal and N nocturnal.

TABLE 2: Phlebotominae sandflies species collected inside human houses in an endemic area for cutaneous leishmaniasis in Costa Rica.

Species/Locality	103	114	202	212	404	416	513	525	0*
<u>W. rotundipennis</u> **		X	X	X	X		X		5/8
<u>W. hertigi</u>							X		1/8
<u>Lu. cruciata</u> **	X		X	X	X	X	X	X	7/8
<u>Lu. gomezi</u> **	X	X		X	X	X	X	X	7/8
<u>Lu. trinidadensis</u>		X		X	X	X		X	5/8
<u>Lu. sanguinaria</u> **				X		X	X	X	4/8
<u>Lu. shannoni</u> **			X	X	X	X	X	X	6/8
<u>Lu. youngi</u> **			X	X	X	C		X	5/8
<u>Lu. ylephiletor</u> **		X	X	X	X	X	X	X	7/8
<u>Lu. serrana</u> **	X	X	X	X	X	X	X	X	8/8
<u>Lu. longipalpis</u> **				X	X	X		X	4/8
<u>Lu. ovallesi</u> **						X	X	X	3/8
<u>Lu. pia</u> **	X		X		X	X		X	5/8
<u>Lu. zeledoni</u>			X		X	X	X		4/8
<u>Lu. runoides</u>				X					1/8
<u>Lu. rosabali</u>						X	X		2/8
<u>Lu. gorbitzi</u>							X		1/8
<u>Lu. atroclavata</u>				X		X		X	3/8
<u>Lu. bispinosa</u> **							X	X	2/8
<u>Lu. odax</u>								X	1/8
<u>Lu. vespertilionis</u>						X	X		2/8
Number of Species	4	5	8	12	11	16	13	15	
Anthropophilic sps**	4	4	6	9	9	11	8	12	

*Occurrence: Defined as the number of localities in which a species occurred divided by the total number of localities. ** Based on Zeledón et al. 1985.

Table 3 shows the species of sandflies collected in the peridomiliary area; it is obvious that a greater number of species is collected outside than inside human houses. As shown in this table, Lu. cruciata (8/8), Lu. gomezi (8/8), Lu. shannoni (8/8), Lu. youngi (7/8), Lu. ylephiletor (8/8), Lu. serrana (8/8) and Lu. pia (7/8) are the most commonly distributed species outside houses.

Murillo and Zeledón collected phlebotominae sandflies mostly in Cangrejal (Tiquires, Naranjal, Ceiba Oeste, Gravilias, Mesa, Ceiba Este, Escuadra), San Ignacio (Chirracá, San Luis), Guatíl (Ococa, Cruz) and Sabanillas (Uruca, Sabánas) (Murillo and Zeledón, 1985). We have collected specimens in three of these localities: Ceiba Este, Chirracá and Ococa. There were significant differences on species composition between their findings and ours on locality (404) Ceiba Este; however, these differences may be explained because they do not restrict themselves to the house as sampling unit.

TABLE 3: Phlebotominae sandflies species collected in peridomiliary areas of human houses in an endemic area for cutaneous leishmaniasis in Costa Rica.

Species/Locality	103	114	202	212	404	416	513	525	0*
<u>W. rotundipennis</u> **		X	X	X	X	X		X	6/8
<u>W. hertigi</u>							X		1/8
<u>Lu. cruciata</u> **	X	X	X	X	X	X	X	X	8/8
<u>Lu. gomezi</u> **	X	X	X	X	X	X	X	X	8/8
<u>Lu. trinidadensis</u>				X		X	X		3/8
<u>Lu. sanguinaria</u> **			X	X	X	X			5/8
<u>Lu. shannoni</u> **	X	X	X	X	X	X	X	X	8/8
<u>Lu. youngi</u> **	X	X	X	X	X	X		X	7/8
<u>Lu. ylephiletor</u> **	X	X	X	X	X	X	X	X	8/8
<u>Lu. serrana</u> **	X	X	X	X	X	X	X	X	8/8
<u>Lu. longipalpis</u> **			X		X	X	X	X	5/8
<u>Lu. ovallesi</u> **	X			X	X	X	X	X	6/8
<u>Lu. pia</u> **	X	X	X	X	X	X		X	7/8
<u>Lu. hartmanni</u> **					X				1/8
<u>Lu. zeledoni</u>				X	X	X			3/8
<u>Lu. runoides</u>		X	X	X					2/8
<u>Lu. rosabali</u>				X	X				3/8
<u>Lu. gorbitzi</u>							X		1/8
<u>Lu. atroclavata</u>				X			X	X	3/8
<u>Lu. bispinosa</u> **				X				X	3/8
<u>Lu. vespertilionis</u>					X		X		2/8
<u>Lu. barretto</u>				X			X		1/8
<u>majuscula</u>									
Number of Species	8	9	11	16	15	13	14	13	
Anthropophilic sps**	8	8	10	10	12	11	9	12	

*Occurrence: Defined as the number of localities in which a species occurred divided by the total number of localities. ** Base on Zeledón et al. 1985.

Occurrence data show how spread is the geographical distribution of a particular species; from the data it is possible to observe highly distributed species such as Lu. serrana, Lu. ylephiletor, Lu. cruciata, Lu. gomezi, Lu. shannoni, Lu. youngi and Lu. pia and poorly distributed species such as Lu. hartmanni, Lu. gorbitzi, Lu. barretto majuscula and War. hertigi.

Inside/Outside Capture ratio: The house should be a physical barrier between the domiciliary and the peridomiliary environment; it is a common control method to screen windows and doors to improve the quality of houses and avoid arthropod infestation. For the purpose of this analysis we assume that the occurrence of one infected individual of phlebotominae vector is enough to transmit disease and then the occurrence of a species is recorded when at least one individual is captured.

We defined I/O Ratio as the ratio between the number of species captured inside by the number of species captured outside; I/O Ratio antropophil. is the I/O ratio corrected using only known anthropophilic species and $I = O$ when the number of species captured inside and outside houses is the same. It is clear that $I = O$ give a I/O ratio = 1. This parameters will be used to indicate the utility of house characteristics to avoid arthropod infestation.

As shown in Figure 1 there is a greater number of species collected inside houses in localities 416 and 525; this difference approach 1 when the I/O ratio (defined as the ratio between the number of species collected inside and those collected outside) is referred only to species previously reported in the literature as anthropophilic. It is interesting to observe that, for locality 416, the abundance of phlebotomines inside houses (165) is three times greater than the abundance outside (52) which also could be related with house characteristics.

These type of data will be more meaningful when we compare between houses using the case-control design as previously used (Rojas et al. 1988; Rojas 1991).

Female reproductive status: It is necessary to record the reproductive status of specimens collected to suggest a relationship between phlebotominae life cycle and human houses. Most phlebotominae sandflies are anautogenous and a blood meal is necessary to complete each gonotrophic cycle and, also, to become infected and transmit leishmaniasis. Phlebotomines do not flight too far after a blood meal, a blood meal is necessary to produce eggs and gravid females can be easily differentiated. A survivorship index has been developed for vector populations based on this type of information from resting populations (Colless, 1958); better methods have been used with phlebotominae sandflies to grade by age group and parity but these methods have been standardized in a reduced number of species (Magnarelli et al. 1984)

We found fed and gravid females in collections made from each locality studied (Fig 2); it was interesting to observe that the greater number of gravid females was collected in (416) Linda Vista and the smaller in (114) Agua Blanca. However, the greater number of empty females was captured in (212) Hondonada.

In Figure 3 we show the distribution of not empty females by species; it is clear that the species that show the greater number of gravid females, Lu. serrana (30), Lu. longipalpis (08), Lu. ylephiletor (18), Lu. gomezi (06), Lu. shannoni (41), Lu. youngi (29), Lu. cruciata (05) and Lu. sanguinaria (46) are known to feed on humans. A higher proportion of Lu. serrana, Lu. longipalpis, Lu. ylephiletor and Lu. gomezi than to any of the other species was found gravid inside houses.

Fig 1: Phlebotominae species, inside/outside capture ratio, in eight localities of an endemic area for cutaneous leishmaniasis in Costa Rica (see materials and methods for locality codes).

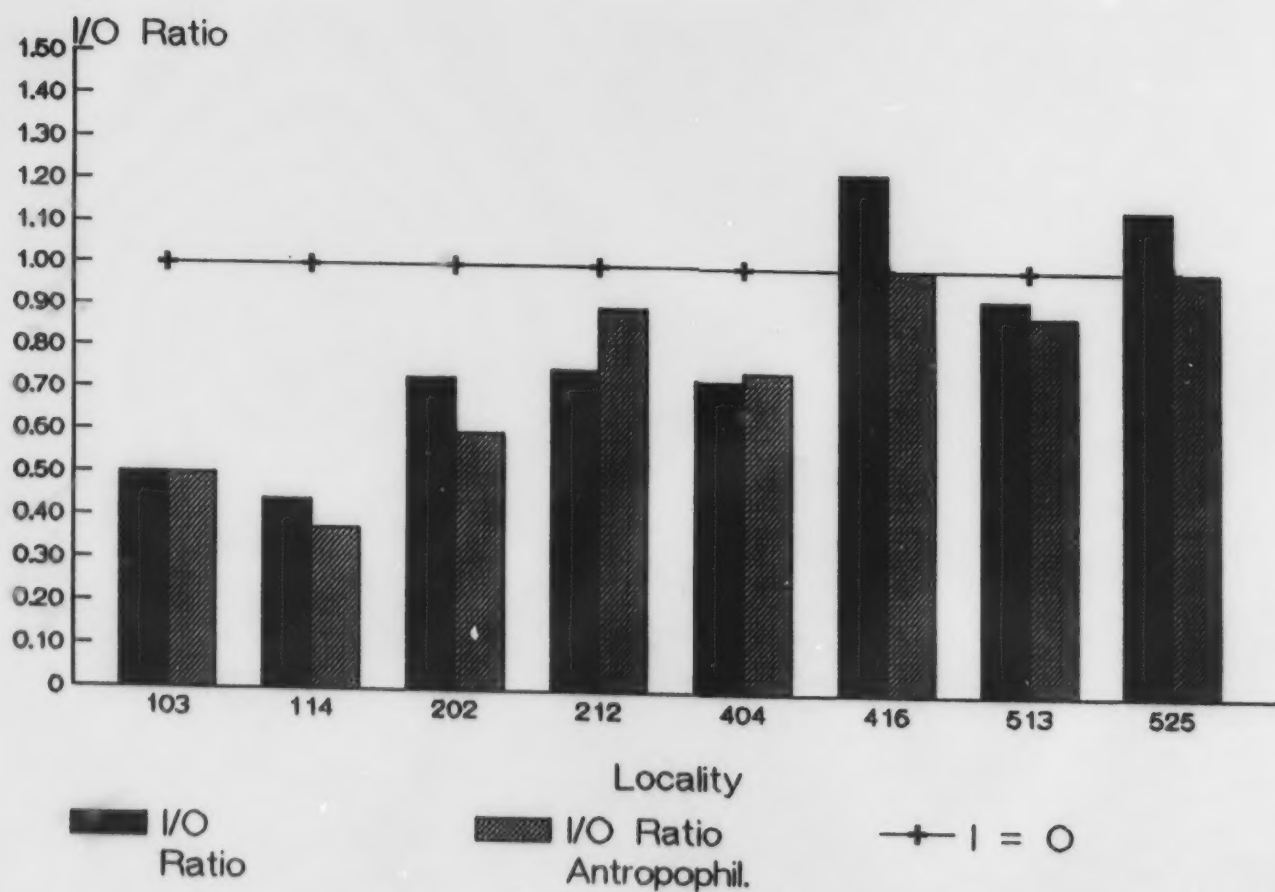


Fig 2: Physiological status of female phlebotomines collected from houses in an endemic area for cutaneous leishmaniasis in Costa Rica (by locality; see materials and methods for locality codes).

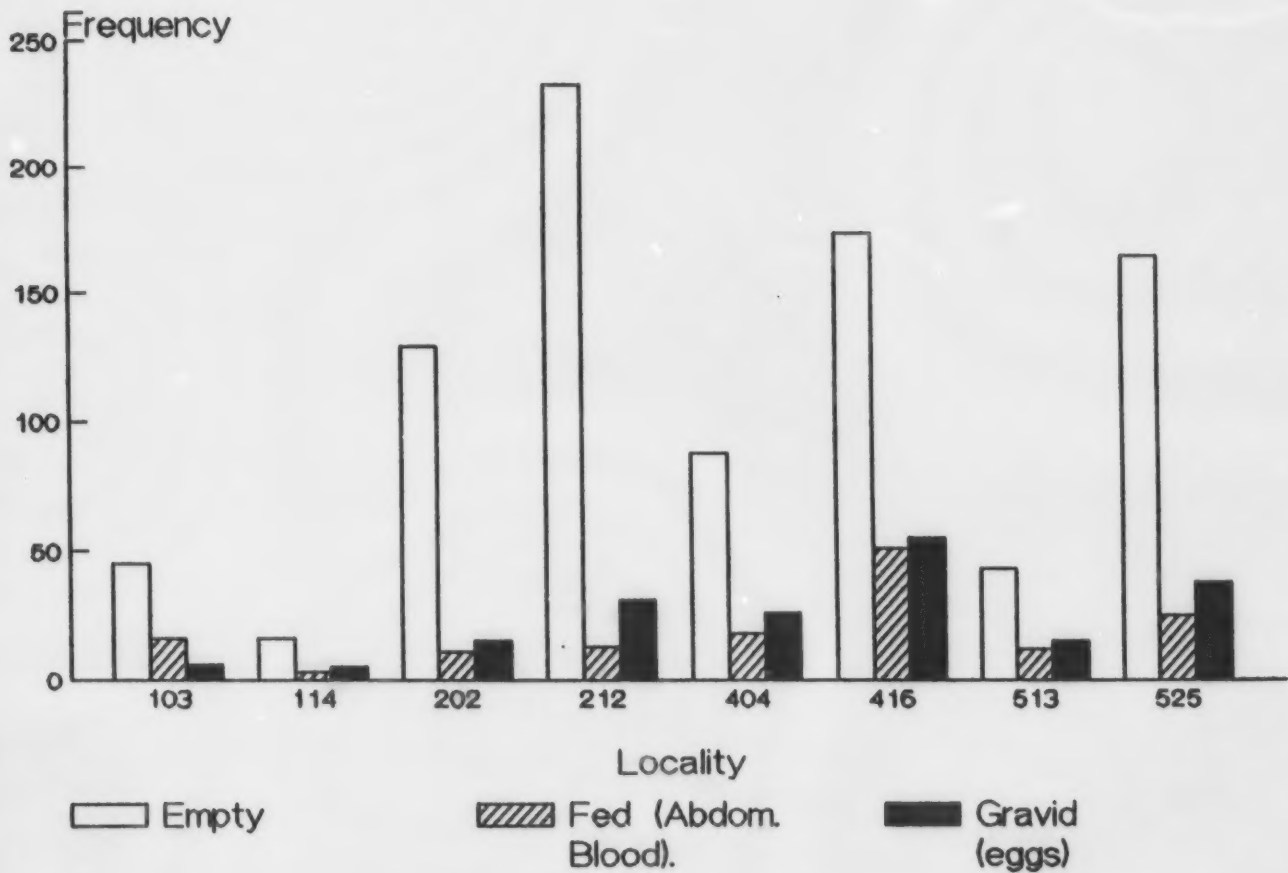
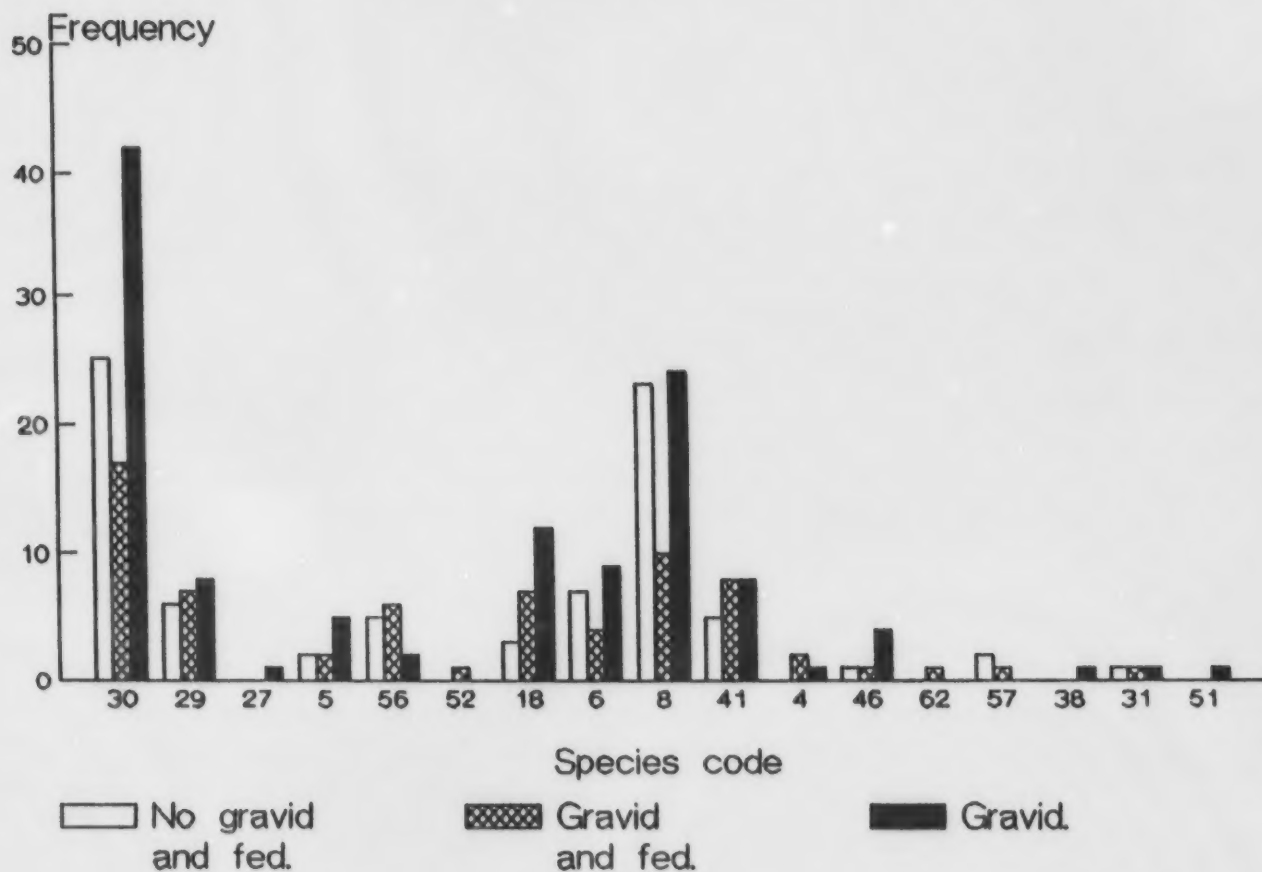


Fig 3: Physiological status of female phlebotominae collected from houses in an endemic area for cutaneous leishmaniasis in Costa Rica (by species; see materials and methods for species codes).



Discussion

Phlebotominae sandflies have been identified as vectors of leishmaniasis in many areas of tropical America. Originally associated with forested and selvatic areas, they have adapted themselves to a man made environment such as coffee plantations and cultivated areas in Acosta where primary forested areas have been destroyed.

The human house in this situation may represent shelter and food to the flies; it is normally provided with humid - shadowed areas and associated with domesticated animals such as pigs, chickens, dogs, cattle which may be used as alternative hosts (Rojas et al. 1988; Rojas 1991). Phlebotomines are not specific on their selection of host and human exposure may be a factor on disease transmission; it has been shown in this paper that in some areas the house do not represent a physical barrier for phlebotomines and even it is possible to obtain a 1:1 relationship between anthropophilic species collected inside and outside houses.

Other aspects besides phlebotominae abundance, species composition, spatial distribution, I/O ratio, female reproductive status are being studied; these are blood meal source, seasonality and resting places dynamics.

In this paper, we have cited a number of species collected on human houses and we have indicated a possible relationship between houses and phlebotomine life cycle. Further information is being produced as part of an epidemiological study on "Risk Factors associated to cutaneous leishmaniasis in Costa Rica" and will be presented on future communications analyzed dividing houses in cases and controls to evaluate differences.

Transmission of cutaneous leishmaniasis is a multifactorial problem; risk factors associated with disease should be identified using epidemiological quantitative tools. Once these factors are known we will conduct field experiments to demonstrate that our recommendations are useful to decrease the incidence of this disease.

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III. Anthropological Input for Epidemiological Research on Cutaneous Leishmaniasis Risk Factors

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Introduction

The objective of this paper is to discuss some of the several ways in which anthropological, qualitative observational input and derived insights are already contributing to sharpen epidemiologic risk factors analysis. This contribution takes mainly the form of a methodological control on the deductive passage from epidemiologic hypothesis to observations; methodological control on the inductive passage from data to empirical summaries is also important.

Child Behaviour Study

An important set of risk factors for cutaneous leishmaniasis, as defined by previous epidemiological work, can be said to be behavioral in nature (Rojas et al., 1988; Rojas 1991). Children aged nine and under constitute an especially prone group in the particular context of Acosta county, where there is a strong suspicion that transmission is occurring domiciliary or peridomiciliary (Rojas et al. 1988).

Thus, the behaviour of this age group has been closely observed, providing a very first recording of this kind of information currently under analysis. Documenting child behaviour patterns in the predetermined setting (houses and their environs) is expected to help identify the role of these behaviours in increasing or decreasing the risk of becoming infected with leishmaniasis.

For this observation, anthropological techniques were used in building up appropriate recording instruments. First, the project's field assistants performed a considerable number of "candid" observations of children with which they were already familiar (so that their activity would be less affected). The only instruction given was to record on a notebook everything that happened to the child in periods of 40 minutes to three hours.

These written records, qualitative in the highest degree, were later analyzed in order to quantify and establish the hierarchy of their referents, actions and modifiers. The sorted listing, still very long, was discussed with the epidemiologist bearing in mind the objectives proposed and the provisionally defined risk factors, until a manageable set of variables was deduced.

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The variables so constructed were put on the columns of a spreadsheet; first row is used to note the time at the beginning of the observation and the codes for the initial status of each variable. Each time that one of these changed, a new row was used to note the time and new variable status.

In practice, this is a very easy and accurate procedure, allowing analysis based on sorting and grouping variables as well as on summing the total time spent at each status during the observation period. Later a comparison will be made between cases and controls.

Child behaviour observation was made by previously trained project personnel on one child per house, using a random subsample of 27 houses comprising both cases and controls. This was done twice a day, at supposed peaks of vector's biting activity (three hours early in the morning, starting at dawn; three hours late in the afternoon, finishing just after dusk), on three alternate days.

The whole procedure was performed twice in the rainy season and once in the dry season, because some variation in children activities is anticipated due to weather and school work (dry season roughly corresponds to school holidays).

A different recording form was designed to be used by the mothers of these same children. Each variable was assigned a different sheet, and within one sheet categories were illustrated by simple drawings in a row; columns stand for rough time periods of half an hour. Mothers are expected to mark the cross-space if they actually observe their child in the situation described by the drawing, and leave it blank if they do not. This observation was performed on the same days as project field assistants plus on the alternate days.

This procedure turned out to be easily followed even by illiterate women, though this situation could be different in other cultural settings, where close attention should be paid to the vocabulary and grammatical structures of the instructions. Simple as they are, drawings should be discussed beforehand with local people and modified accordingly, as was done in Acosta.

Both types of observation were not thought to be comparable, because of the differences in observer, instrument, scale and time intervals. Nevertheless, observation by mothers has shown to be a better input source than thought previously. Independent of its information value, the procedure has been a highly valuable and recommendable one in order to involve community participation and interest in the project.

Unexpected insight arose as mothers recurrently made the "mistake" of marking at the same time in two rows that had been thought to be incompatible: "My child is inside the house" and "My child is on the cleared space around the house", and also "My child is inside the house" and "My child is under the house" (a large number of houses being built on stilts).

These apparent contradictions were discussed with the mothers, leading to the realization that the two spaces mentioned are clearly regarded by them as extensions or even as parts of the house itself, as far as child activities are involved. This concept changed depending on the child's sex and age.

Clearly, it appeared that for the purposes of the risk factors study it is important to understand the domestic and peridomestic space as people see and feel it, not only as it is epidemiologically defined. This issue was included among others in a more specifically anthropological exploration of risk factors supported by a TDR small grant through Central University of Venezuela's Social Research Laboratory.

Cross-Control on Observers' Bias

An epidemiological survey is being carried on using a comprehensive questionnaire consisting of 65 items, mostly questions to be asked but also observations to be made and measurements to be taken. Again, the anthropologist played an important role in the instrument design, including checking vocabulary and grammar details with local population.

As stated in the original proposal, a cross-control for observers' bias was performed at a relatively early stage of the research for the epidemiologic questionnaire and corresponding field measurement and observation procedures.

The whole survey was repeated on a random subsample of 16 houses, at an average interval of some three months after it had first been done (this was expected to reduce respondent's recall and fatigue biases, but in our experience the period of time was too short for that to matter). Interviewers were interchanged so that each one could act as a control for the other.

Simple comparison of frequencies seemed to indicate some possible problem with the following: instrument's design, coding, instruction sheet, respondent's bias and last but not least, interviewers' bias for which the procedure was primarily intended, as well as the interviewer's training.

This was a quite worrying result, so a critical analysis was readily carried on in order to localize, detail, evaluate and hierarchically sort out the differences which were found. The objective of analysis was to assign each difference to one or more of the causes enumerated above, so that these could be corrected as much as possible.

The largest number of differences affected the following items:

Number 24 (interrogation): "I will repeat you the whole list of animals⁵, in order to be sure none has been skipped and to know how many they are" (question "how many?" is asked each time a category of animals is marked).

Number 52 (observation): "With which material are the windows closed or covered?" (observer is to note how many windows are closed with each category of materials and how many are not closed at all).

Number 58 (observation): "What kinds of plants, trees and crops are there around the house? Note usual names" (observer is to note how many are there of each usual name between each category).

Number 65 (observation): "What distance is there between the house and the facilities built around it?" (observer is to measure and note distance between house and each category of facilities built around it).

As it can be seen, first three items involve counts and the last one involves continuous measurement. It was decided to accept all differences within an average of ± 1 standard deviation, and to accept a largest difference in all but two of the sixteen houses (that is 10.5% rounded upwards). Thus corrected, the test was good as much as observer's bias is concerned. Nevertheless, first results pointed out important features to be taken into account and clarified by closer qualitative observation (also under TDR's small grant).

The recall question on animals (as possible reservoirs, or as vectors' attractors) led us to realize that differences in their numbers can indeed be real within a three month period, mainly if a seasonal weather change is also involved. Greater variation was observed in higher reproduction rate animals, dogs and domestic fowl.

The number of domestic fowl was found to be more accurately reported by women. The number of dogs, pigs, milking cows, newborn calves and caged birds did not seem to show a difference (note that all these animals are at least partially peridomestic). Men were found to more accurately report numbers of all other animals, which are generally kept at a greater distance from the house and pertain to masculine tasks.

Potential periodical variations were found to be:

- a decrease in number of domestic fowl due to Holy Week (large family and community celebrations, Roman Catholic traditional ritual interdiction of red meat consumption during this time, drying up of corn supplies for feeding fowl);

⁵ This list recalls the animals that have just been classified in the preceding questions and uses the following categories: i) come or stay around (or under) the house during daytime; ii) come or stay inside the house during daytime (even for short periods); iii) come or sleep around (or under) the house during nighttime and using to sleep inside the house during nighttime (even for short periods).

- a decrease in the number of pigs due to Christmas and New Year (large family and community celebrations, pork meat consumption as a choice dish - mainly in the form of "tamales", which also employ large quantities of corn);

- a variation in the numbers of bovines (milking cows and their newborn calves as it has been indicated, but also oxes for sugarcane grinding, a masculine task performed close to the house).

Differences in window covering materials concerned mostly a special kind of window, described by one of the observers as "Closed with wood" and by the other as "Closed with a material other than the above". For this, the latter is correct, for if in this kind of window the material employed is wood, it generally consists of narrow slabs in the form of a lattice.

There is usually one window of this kind per house (when there is one), placed behind kitchen sink. It is a usual location for women and young age children. The space under it is always humid and bears food rests carried by washing water. Such a water and food source is very attractive for animals.

In connection with this, it has later been observed that clothes washing is usually not performed in this same sink, but near the water source which, incidentally, is regarded by people as the main locale for cutaneous leishmaniasis transmission (a biologically sound folk-assumption, which has not been epidemiologically demonstrated or tested).

With regards to plants, trees and crops around the house, taken into account as selective sugar sources for vectors (Sclein and Yuval 1987), a relatively high variation was expected. It should be noted that practically no rice, beans nor corn crops exist around houses. Analysis of differences within this question has led to further anthropological work on: seasonal variations of blooming and fruit-bearing periods of most extended varieties; cutaneous leishmaniasis folk-treatments attributed to certain plants; the aesthetic and other criteria for the choice and combination of plants within a garden by women; the influence of fashion and novelty phenomena concerning the changing of varieties; and informal observation of gardening behaviour patterns.

A grouping of the most popular plant varieties by their potential as a food source for vectors been proposed and will be carried on jointly by an anthropologist and entomologist.

Finally, analysis of the question concerning facilities built around the house has led to more detailed questioning and observation on the various folk definitions and changing uses made of each facility. A tendency toward separate male and female spaces between and within facilities, as well as inside the house, has been observed and demands more clarification.

Discussion

Two ways in which anthropological data and techniques can be used to assist evaluation of epidemiological data on cutaneous leishmaniasis risk factors have been presented.

Anthropological input into epidemiological research on risk factors for cutaneous leishmaniasis has been shown to be important for designing culturally-sensitive questionnaires, interview instruments and children's behaviour recording instruments, as well as for developing an awareness on how some measurement techniques are not always the most appropriate.

Methodological issues raised by combining the disciplines of epidemiology and anthropology to determine the actual appropriateness of risk factor measurements in the group being studied have been showed to be potentially relevant to the further development of control and prevention initiatives. This is especially true for those risk factors that stem from the behaviour of individuals or from alterable aspects of human domiciliary and peridomiciliary spaces.

Through anthropology greater attention is been given to the interrelationships of risk factors, and to understanding the host-environment interactions in the unique social and cultural group of Acosta peasants. On the other hand, quantitative epidemiological measurements have directed qualitative anthropological research by pointing to patterned relationships between variables that require further in-depth analysis.

The importance of this kind of research collaboration for cutaneous leishmaniasis control and prevention lies in providing data vital to the successful implementation of culturally-appropriate actions or programs. Without significant input from anthropology, in terms of analyzing the context and determinants of individual risk behaviour, epidemiological data alone would seemingly provide only an incomplete basis for the design of sound control and prevention strategies.

Grassroots anthropological insights into the community and its culture have so been shown to enable the evaluation of social inputs in terms of their relative importance as determinants of cutaneous leishmaniasis transmission and later in its control and prevention through measures that can be integrated into the various lifestyles of the community.

The application of anthropological knowledge needs to be directed primarily towards socio-cultural solutions rather than medical interventions. Risk intervention should be redirected to the socio-environmental contexts, which requires political and economic solutions. This paper has clearly stressed that anthropological input can be useful in the continuous monitoring of epidemiological data quality and collecting procedures, which is essential to ensure that the control and prevention measures that are proposed and implemented on the basis of these data will be highly effective.

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Vector Blood Meal Sources and Transmission Studies on Andean Leishmaniasis¹

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Summary

The relationship between the sandflies, Lutzomyia peruensis and Lu. verrucarum, and their bloodmeal sources in the domestic environment was investigated in an attempt to explain the patterns of leishmaniasis transmission in an endemic village in the Peruvian Andes. The blood meal analysis precipitin test was used to determine the source of ingested blood; and Polymerase Chain Reaction technique was applied for Leishmania detection in sandflies. These were caught indoors with CDC light traps and outdoors with protected human baited Shannon traps; collections were performed monthly during one year (1990-1991) in Chaute (Lima, Peru), at an altitude of 2500 metres.

Sandflies enter houses continuously, using these as feeding places; human blood is the most important source of blood indoors. In the rainy season (January to March) the sandfly invasion of houses increases, and engorged sandflies also abound. One specimen of Lu. verrucarum was found infected with Leishmania in the anthropophilic collections. The data recorded suggest that the sandfly behavior (highly anthropophilic and endophilic) allows prolonged periods of man-sandfly contact; the risk of infection with Leishmania indoors is high for these reasons. Assays to study the avoidance of sandfly bites with Eucalyptus oil were performed with positive results.

Introduction

This study was carried out on sandfly behavior and transmission aspects of andean cutaneous leishmaniasis called Uta; Leishmania (Viannia) peruviana is the parasite causing the disease with lesions usually occurring on uncovered parts of the body. The sandfly species vector is not clearly identified yet. Lutzomyia peruensis and Lu. verrucarum which are widely distributed and abundant in the leishmaniasis areas in Peru are probably both involved in the transmission. Lutzomyia peruensis was found infected with Leishmania peruviana (Perez et al. 1991). The disease remains endemic in most of the valleys of the Andean Cordiller at altitudes from 1800 to 3000 metres above sea level.

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Objectives

The main objectives of this study were:

- 1) To determine the relative importance of man and domestic animals (which live in close proximity to man) as blood sources for the sandflies of Chaute (Lima, Peru) -- an endemic area for Andean Cutaneous Leishmaniasis.
- 2) To detect the presence of Leishmania infection in Lutzomyia species of domiciliary and peridomestic environments from Chaute through the Polymerase Chain Reaction (PCR) technique, to try to ascertain the place in which transmission occurs.

The Study Area

The village of Chaute is located 70 km east of Lima (the capital city) in the Rímac Valley, at 2500 metres above sea level in the western slopes of the Andean Cordiller. Low vegetation (grasses, herbs and shrubs up to 80 cm high) cover the slopes, whilst trees are confined to the rivers and small brooks. Two defined seasons occur during the year: a "dry" season from April to December and a "rainy" season from January to March with copious rain. The average temperature during the study was 13.85°C; monthly fluctuations of temperature were not significant for sandfly activity. The relative humidity varies according to the season; being higher in the rainy season (up to 94.27 % in December), and lower in the dry season (down to 21.07% in July). Dessication of small organisms such as sandflies is high, and this limits their activities and populations.

The main human activity in Chaute is agriculture; fruits (apples, chirimoyas, peaches, avocados) are the common crops in the valley. Small numbers of cattle and goats or sheep are also bred. Fifty-seven donkeys are used for carrying fruit from the croplands to the village; during the night these animals stay in yards around the houses within the village.

Chaute has 280 inhabitants, 75% of whom show Leishmania (Uta) scars, normally in uncovered parts of the body. Leishmania (Viannia) peruviana is the parasite causing the disease in the area; it was identified from patients affected with Uta through isoenzyme characterization (Perez et al. 1991). There is a primary school with 58 students, 15 of them with scars or active lesions of Uta. Up to six young children had active Uta lesions on their faces during the study period, and these children stay permanently in the village.

Houses are usually built with unfired mud bricks; the roof is a combination of canes and mud covered with tiles as a protection against the rain. Some new houses are being built with material from the Coast. Houses provide suitable conditions of temperature and relative humidity for sandflies daily entrance. The houses are usually

divided into two rooms which used for all purposes (bedroom, dining room, kitchen, grain storage). Small domestic animals (guinea pigs, cats) are usually found indoors during the night in the same rooms with humans. This is a common practice in Andean villages. Dogs sleep around the outside of houses as guards.

Methods

Sandfly collection methods: Sandfly collections were performed five times each month from June 1990 to May 1991. The methods used were described by Perez et al. (1988) for Andean environments, a brief description of them follows:

Anthropophilic collections: These were carried out using a Shannon trap with protected human bait operated from 1700 to 2400 hours. The trap was placed 30 meters away from the village boundaries. The sandflies attracted to the bait, entered the trap and rest on the inside walls where they were captured with manual aspirators, and separated every hour. Temperature and relative humidity were recorded for the collecting period.

Intradomiciliary collections: These were performed in three houses within the village using CDC light traps operated from 1800 to 2230 hours. The traps were placed in the main room of the houses with their inhabitants inside.

Searching in diurnal resting sites: Searches were conducted with manual insect aspirators in holes and rock crevices around the village.

Bloodmeal Analysis: Precipitin tests (BAPT) were performed with specific antisera on common domestic animals in Chaute. The BAPT test is based on the ability of antibodies (antisera) to precipitate antigens (animal sera). The formation of a lattice of cross-linked antibody-antigen complexes, too large to remain in solution, appear as precipitation bands in the positive reactions. To carry out the BAPT tests, commercial antisera (SIGMA) and antisera produced in our laboratory were used. The reaction was developed in non-heparinized micro hematocrit tubes (Tempelis and Lofi 1963; Christensen and Vasquez 1981; Vásquez, personal communication). Supernatant of rehydrated engorged sandflies as an antigen were taken in a microhematocrit tube, then the antiserum to be tested was added and the tube was sealed. The precipitation band of positive samples was observed after 10 to 50 minutes at room temperature.

The PCR technique was used to detect *Leishmania* DNA in wild caught sandflies. The primers MP1-L and MP3-H used for the PCR procedures were developed in our Institute with *Lu. peruviana* strains (López et al. 1990). These primers are directed to a 65 base pair (bp) consensus sequence (CS) present in minicircles of kinetoplast DNA (kDNA) specifically from *Leishmanias* of the *L. braziliensis* "complex". Sandfly materials were tested in a pool of ten from the same species and catch or source of blood meal. Each sandfly was first individually treated for DNA extraction, a fraction of these were

put together with others to complete the pool. Once a positive pool was found, the original material of the ten sandflies were tested separately to determine the number of positives from each pool.

Results and Discussion

Four species of sandflies have been identified from our collections in Chaute, they are: Lutzomyia noguchii, Lu. peruensis, Lu. verrucarum and Warileya phlebotomanica. Lu. noguchii and Warileya phlebotomanica were very rare; only 3 specimens of both were collected. A total of 6,601 sandflies, 522 (7.9%) males and 6,079 females (92.01 %) have been collected in Chaute. Lu. peruensis and Lu. verrucarum were recorded throughout the year in both anthropophilic and intradomiciliary collections (Table 1).

Table 1: Sandfly Collections in Chaute

	INTRADOMICILIARY				ANTHROPOPHILIC			
	Male	Female	Engrd	Sub Total	Male	Female	Engrd	Sub Total
<u>Noguchii</u>	0	0	0	0	1	1	0	2
<u>Peruensis</u>	42	251	82	293	94	894	30	988
<u>Verrucarum</u>	253	2924	1195	3177	132	2008	55	2140
<u>W. Phleb.</u>	0	0	0	0	0	1	0	1
Total	295	3175	1277	3470	227	2904	85	3131

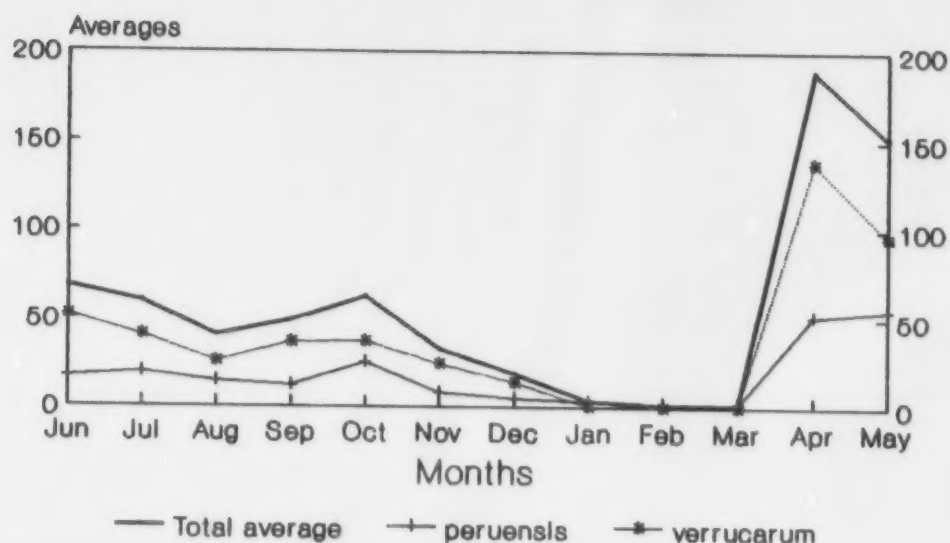
With regards to sandfly anthropophilic activity (Graph N° 1) Lu. peruensis made up 31.55% of these collections. Lu. verrucarum (68.34%) is the predominant species in the area. The largest frequency of sandflies was recorded in April at the beginning of the dry season. Over the following months the frequency decreased continuously becoming almost zero during the rainy season. Both species Lu. verrucarum and Lu. peruensis showed the same trends throughout the year, but Lu. peruensis dominated during the three months of the rainy season.

Sandfly attraction to man was found to be determinant for contact. The sandflies were shown to move to where man is for biting. Such contact can take place when the men return to the village in the night or when they are tending cattle or crops (not frequent in Chaute). There are a few temporary shelters, and isolated houses located on the slopes. The human baited Shannon trap was located 30 m from the houses, and when it was operated simultaneously without bait a few sandflies were captured. Sandflies appear to be widely distributed in rural areas and are attracted by the bait.

Anthropophilic hourly activity of sandflies is shown in Graph N° 2. The greatest sandfly activity was recorded from 1800 to 1900 hours, with remarkable occurrence until

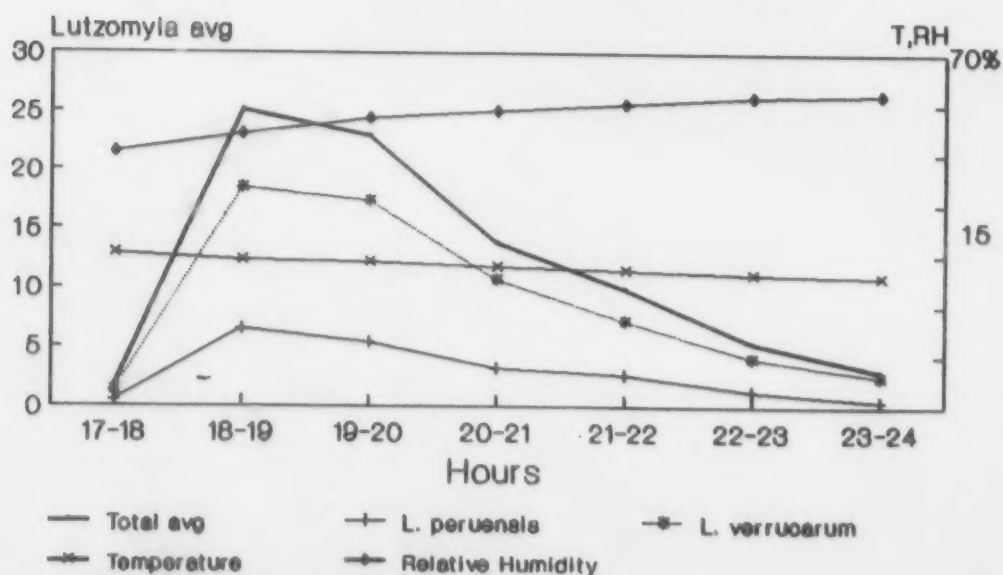
Graph No 1: SANDFLY ANTHROPOPHILIC ACTIVITY

Chaute, Lima, Peru. 2500 m altitude



Shannon trap with human bait

Graph No 2: SANDFLY ANTHROPOPHILIC HOURLY ACTIVITY IN CHAUTE



2000, after which the activity decreased continuously to midnight. The sandfly activity started around 1740 hours for both species which showed the same trends. The relative humidity increased at the sametime sandfly activity decreased, while temperature was almost stable.

Intradomicilliary occurrence (Graph N° 3) of Lu. peruensis was 8.44 % and Lu. verrucarum 91.55 % of those captured. Both species showed the same patterns as the anthropophilic records; the higher occurrence was in May. Lu. verrucarum was the dominant species, Lu. peruensis was almost the 10th part of the total sandflies caught. An increase occurred in January when the rainy season began, probably due to the adverse weather conditions outside driving the sandflies indoors. The almost null records in March are evidence of the reduction of the sandfly population in this month. The endophilic behavior of females of both species is high, although they became more endophilic in the middle of the dry season; occurrence of Lu. peruensis changes markedly in September when it invades the houses more actively.

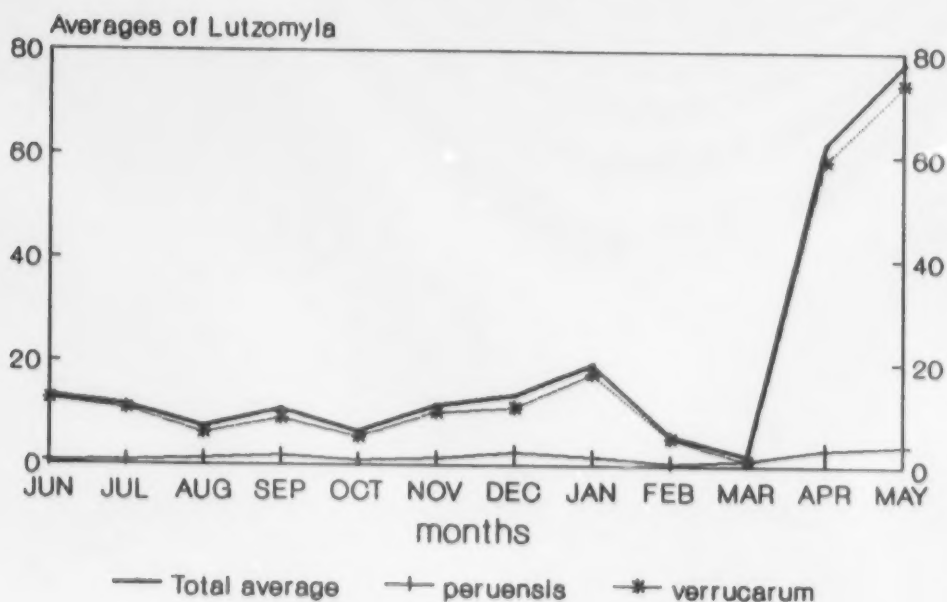
Houses are the main sandfly-man contact place. Entrance of the sandflies is due to their search for diurnal resting sites, but also for feeding. The fact that 8.5% of the sandflies collected indoors were males indicates that the houses are not a resting site, we recorded rates up to 56% of males in similar areas in typical resting sites. As man is in the houses when sandflies are highly active, attraction can be important for house invasion. The cause for this needs to be studied in order to clarify sandfly endophilic patterns. This behavior could be avoided using repellents or spraying insecticides indoors. Early assays were performed with eucalyptus oil as a repellent with positive results.

Searching sandfly diurnal resting sites in the village of Chaute and surrounding areas was not successful. Only a few and small rock crevices and holes were found and searched monthly for sandflies. Only rarely were any sandflies found.

Graph N° 4 shows that 22.55% of the total female sandflies collected were engorged; 2.71% were found in the anthropophilic collections, more frequently in April and May. The already engorged sandflies collected probably need to complete their engorgement or reacted to their attraction to man.

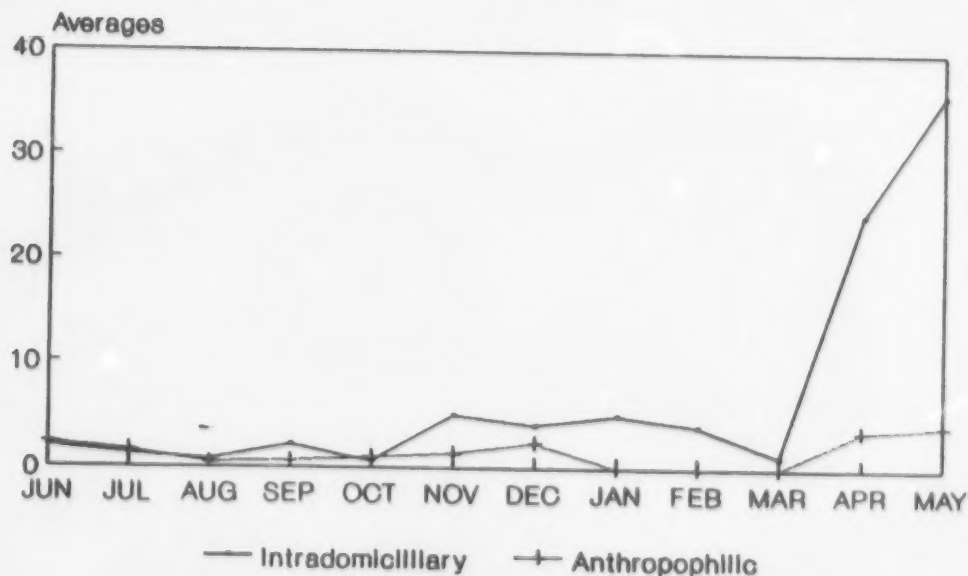
Indoors, 36.8% of the sandflies collected were engorged. Frequency was regularly higher in indoor collections than in anthropophilic collections. A marked increment occurred in April and May once the dry season started. Both species were found engorged at very high rates indoors in February within the rainy season. This was opposite to the anthropophilic records. The sandflies are apparently confined to the houses due to the adverse weather conditions outside which limit their activities. Preliminary results of the sources of 919 bloodmeals identified from 1362 total engorged sandflies, are presented in Table 2.

Graph No 3: INTRADOMICILLIARY COLLECTION In Chaute, Lima, Peru



CDC light traps placed indoors

Graph No 4: ENGORGED SANDFLIES IN CHAUTE, LIMA, PERU Monthly Averages



2500 m altitude

Table 2: Sandfly Blood Meal Sources in Chaute

	HOUSES		ANTHROPOPHILIC	
	No. <u>Peruensis</u>	No. <u>Verrucarum</u>	No. <u>Peruensis</u>	No. <u>Verrucarum</u>
Human	25 (46.29%)	245 (31.25%)	6 (20.68%)	15 (28.84%)
Cat	12 (22.22%)	133 (16.96%)	8 (27.58%)	11 (21.15%)
Goat	2 (3.70%)	70 (8.92%)	0	1 (1.92%)
Dog	4 (7.40%)	104 (13.26%)	1 (3.44%)	4 (7.69%)
Cow	4 (7.40%)	165 (21.04%)	8 (27.58%)	12 (23.07%)
Donkey	4 (7.40%)	12 (1.53%)	1 (3.44%)	1 (1.92%)
Sheep	2 (3.70%)	25 (3.18%)	3 (10.34%)	3 (5.76%)
Guinea pig	0	0	0	0
Horse	0	12 (1.53%)	0	0
Rabbit	0	11 (1.40%)	1 (3.44%)	1 (1.92%)
Rat	0	0	1 (3.44%)	0
Pig	1 (1.85%)	4 (0.51%)	0	0
Mammal	0	0	0	0
Chicken	0	0	0	0
Avian	0	2 (0.25%)	0	2 (3.84%)
Undetermined	0	1 (0.12%)	0	2 (3.84%)
Total	54 (100%)	784 (100)	29 (100)	52 (100%)

With respect to anthropophilic captures, cows and cats were the main sources of blood for Lu. peruensis; human blood follows in preference. Lu. verrucarum was more anthropophilic than Lu. peruensis; cows and cats were their secondary option. Findings of blood from indoor and outdoor sources in the same sandfly of both species suggests they move actively from the rural areas to enter the houses and vice versa in order to find sources of blood to feed on.

In the intradomiciliary captures, although both species showed high preference for human blood (anthropophilic trend), Lu. peruensis were found to feed on man more actively than Lu. verrucarum; cat blood was the secondary preference of both species. Man and cat usually sleep indoors in Andean places and are continuously exposed to sandfly bites. Cows and dogs follow in preference, but mainly for Lu. verrucarum, indicating this species moves to find the cows; dogs are probably bitten while they are guarding the houses in peridomiciliar sites. Lu. verrucarum preference for human blood was high from September to February; cat blood appeared normally low except from June to August in which it was dominant.

The sandflies apparently have no restrictions on feeding on the animal sources of blood which are usually indoors at night; both have large periods of coincidence in that environment when sandflies are active and hosts resting. Men and cats in the houses and cows outside (in the yards) are probably more accessible for biting. A free choice of host

can occur according to sandfly preferences but a succesful feeding will also depend on the activity and movements of these hosts (Edman and Spielman 1988).

The domestic animals cohabitating with people indoors are also important for sandflies as sources of blood; the anthropophilic character is, however, dominant in spite of the number and permanent presence of such animals (e.g. cats, guinea pigs, sometimes dogs).

Cats were shown to be important for sandflies as source of blood in Chaute. They were introduced to the village to control the mice population and these animals are not abundant in other regions in the Cordillera. The permanent presence of cats in houses results in competition with humans as hosts for sandfly engorgement. Human sandfly bites (highest from 1800 to 2000 hours) could be reduced due to the presence of this secondary preference source in the house.

The fact that young children and adults permanently sleeping in the village developed Uta, and the recording of high sandfly bites to man indoors (which was previously thought insignificant), are evidence that Uta transmission can be intradomiciliary.

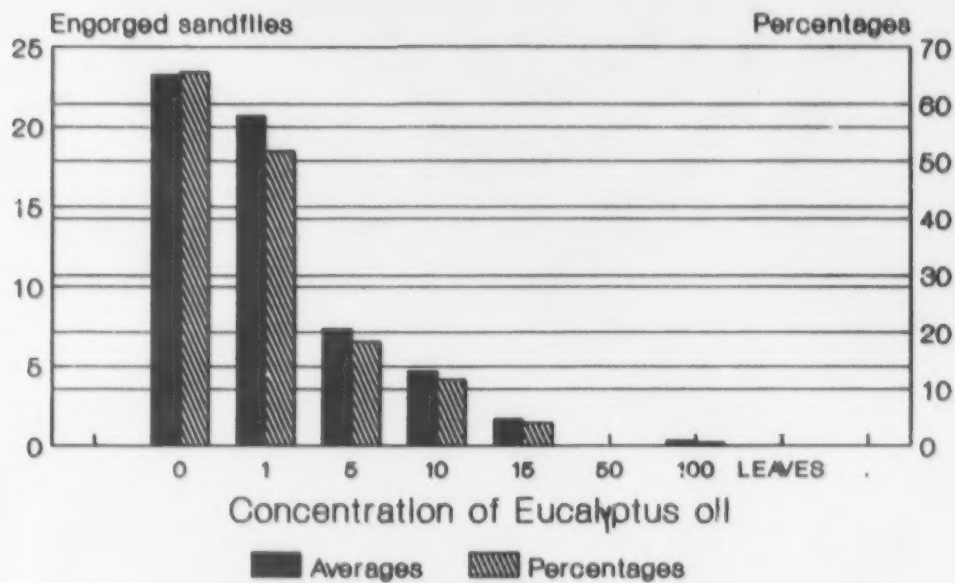
Multiple bloodmeals were found in 129 (9.47%) of the total engorged females; 107 of them indoors and 22 in anthropophilic captures. Higher rates occurred in August within the dry season. In general, bloodmeal from humans, cats and cows were more frequently found; blood from dogs, donkeys and goats were less frequent. Combinations found involving animals in yards and indoors are evidence that the sandflies move from the yards to the houses or viceversa searching for sources of blood. Multiple bloodmeals are usually the consequence of interrupted engorgements.

From a total of 6,079 female sandflies collected in Chaute, 2,350 (1892 Lu. verrucarum and 458 Lu. peruensis) were tested in pools of the same species and catch to detect Leishmania parasites through PCR testing.

One specimen of Lu. verrucarum from anthropophilic collections was found positive to Leishmania (26.abr.90, 1900 to 2000 hours). This is the first time this species was found infected with Leishmania. It obviously does not necessarily incriminate this sandfly species as vector. However, Lu. verrucarum can play an important role in the transmission of the disease in Chaute because of its abundance and close relation to man. The species may also take part in the maintenance of the infection in wild and domestic animals. As the specimen was caught in a rural area which was located very close to the village houses, we do not conclude that the transmission was rural, given the high endophilic character of Lu. verrucarum.

The design of PCR tests in pools of sandflies is a suitable tool for detection of natural infected sandflies in large numbers of insects in a short time. Any sandfly species

GRAPH N° 5. SANDFLY REPELLENCE USING EUCALYPTUS OIL



can get the parasite by feeding on infected hosts. The PCR technique enables the detection of Leishmania in the sandfly despite of the number, stage of the parasite or the capability of the sandfly to transmit it.

The results of using a eucalyptus preparation as a sandfly repellent are presented in Graph N° 5. Concentrations of eucalyptus oil of 0, 1, 5, 10, 15, 50, 100% diluted in 95% ethanol were applied to the whole body of hamsters which were anaesthetized with 0.3 ml of Ketalar. The hamsters were placed into feeding cages and exposed to 40 female sandflies for one hour. Results showed that sandfly engorgement is proportionally inverse to the eucalyptus oil concentration. The more concentrated the eucalyptus oil, the less sandflies feed on the hamster. At concentrations of 50% eucalyptus oil 3 out of 40 sandflies were found dead; at concentrations of 100 % eucalyptus oil the mortality rate of sandflies was 100%. It was observed that sandflies land or bite on untreated skin areas.

Additional assays were performed using green eucalyptus leaves as bed material for hamsters. No sandfly engorgement resulted. More assays should be made to find the best way to avoid sandflies biting humans and entering houses. We suggest the entrance of sandflies to houses could be avoided by creating an atmosphere of eucalyptus scent inside. A continuous source of this smell needs to be placed in the houses, this will be facilitated by the fact that houses are permanently closed even during the day.

Conclusions

The main conclusions of this study which are important to consider in the design of control measures are:

1. Houses are the main sandfly feeding places in Chaute.
2. Human blood is the main source of bloodmeal for sandflies captured indoors in Chaute.
3. The intradomiciliary anthropophilic character of Lu. peruensis is higher than that of Lu. verrucarum.
4. The risk of being infected with Leishmania indoors is high, due to the coincidence of humans and sandflies in time and space.
5. Lu. verrucarum is a probable vector of Leishmania in the study area due to its abundance and contact with man; its role in the transmission of the disease the man needs to be clarified.
6. Odorous eucalyptus oil preparations which were tested showed effective repellent activity against sandflies. The sandflies did not land or bite on treated skin.

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Community and Environmental Risk Factors Associated with Cutaneous Leishmaniasis in Montebello, Antioquia, Colombia

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Introduction

The establishment of measures to control zoonotic diseases such as leishmaniasis requires understanding of the mechanism of transmission within the natural focus of infection. The identification of the parasite species present in the endemic focus, the vector species and reservoir animals are the three basic elements of transmission. Studies of vector population dynamics, which determine the focus of the endemic zone, are of fundamental interest (1).

Delimiting the focus, identifying the population at greatest risk of becoming ill, determining the time of year when infection is most likely to occur and the location, with respect to human dwellings, where humans come into contact with infected vectors, are points that must be addressed to design rational measures of epidemiological surveillance and control.

Pioneering studies such as those of Professor J.A. Rioux have adapted the ecoepidemiological method of analysis for the calculation of risk of contracting leishmaniasis in endemic foci in the Old World (2).

In Latin America, the study of leishmaniasis is complicated by the large number of species of the parasite that affect humans, the variety of species of phlebotomine sandflies and reservoirs, and the diversity of habitats where parasites are endemic, ranging from Amazon forests to deserts, from sea level to Andean mountain valleys and chains, and from rural zones to urban areas in some cities (3,4,5).

The present study of an endemic focus of cutaneous leishmaniasis caused by *Leishmania braziliensis* was carried out in Montebello, Colombia, a small town in the Andes Mountains. Our goal was to determine the epidemiological risk of contracting leishmaniasis within this focus.

The main objective of the study was to establish the biological basis of the modes of transmission of cutaneous leishmaniasis in Montebello, by determining the prevalence of the disease, identifying the species of parasite, the vectors and reservoirs present, and relating these findings to ecological characteristics of the region.

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Materials and Methods

Description of the zone studied

Montebello is a town located 50 km from the city of Medellin, Colombia. Data were collected from the surrounding rural area from August 1986 to January 1989. Montebello is located at 5°, 57' north and 75°, 32' west on the west slopes of the Andes Mountains, at an elevation ranging from 800 to 2200 metres above sea level (m.a.s.l.) (Fig. 1).

The region has been inhabited since the beginning of the nineteenth century by farmers; the main cash crops are coffee and fruit. The current population of the rural area is 2,101 inhabitants.

In 1984 there was an outbreak of cutaneous leishmaniasis caused by *L. braziliensis* in Campoalegre and La Merced boroughs on the outskirts of Montebello (6). The epidemic followed the arrival, in June 1983, of two persons with a total of 11 active cutaneous leishmaniasis lesions between them. Two months later, the first cases were identified in persons who had not travelled beyond these boroughs. In subsequent months there was an exponential increase in the number of cases, which had reached 56 by January 1984 (26% of the population in these boroughs). Patients ranged in age from 8 months to 69 years; 32 (57%) were males, and 24 (43%) females. The greatest number of cases was observed in patients aged 15 to 44 years, however, the highest incidence (number of cases per age group) was found in children younger than 4 years (43% and 50% in males and females respectively). Treatment with antimony derivatives brought the epidemic under control. Since 1984, sporadic cases have been reported to the Colombian health service (7).

Study population

To calculate disease prevalence we actively searched for cases through the study period. Lesions compatible with cutaneous leishmaniasis were sampled with a smear, and the exudate was cultured on NNN medium; in addition, biopsies were taken from the margin of the lesion. Indirect immunofluorescence and Montenegro tests were performed.

To determine the degree of human contact with the parasite, we administered the Montenegro test via automatic syringe (Dermojet) to a population of schoolchildren in the study zone.



Figure 1. : The Montebello, COLOMBIA
site of a cutaneous Leishmaniosis focus

Phlebotomine sandfly fauna

We used seven capture points located at different elevations between 800 and 2200 m.a.s.l., and separated by a distance of approximately 200 metres. During 30 months, the authors visited the zone every 15 days to collect phlebotomine sandflies, which were captured as follows:

- a) **Paper traps impregnated with castor oil:** Sheets of white bond paper measuring 20 by 20 cm were impregnated with castor oil and placed on bamboo stands, as described by Rioux (8). At least 20 such traps per station and per visit were placed in domestic (corridors and rooms), peridomestic (independent buildings that housed domestic cattle and fowl, located within a radius of 100 m from the human dwelling), and sylvatic sites (caves, rock crevices and tree hollows located more than 100 m away from the human dwelling). The traps were replaced every 15 days. All phlebotomine sandflies captured were detached from the traps with a small brush soaked in 90% alcohol, and preserved in alcohol for subsequent clearing and identification. We determined the species present, their relative density per square meter of oiled paper in each dwelling, at each capture point and at each season of the year.
- b) **CDC type light traps, Shannon traps and oral suction:** To determine the hours of peak activity of each species (circadian variation) we used CDC type light traps at domestic and peridomestic sites. At the same time sandflies were captured by oral suction from Shannon traps and resting sites. Phlebotomine sandflies were identified according to the scheme proposed by Lewis et al. (9).

Reservoir studies

In areas where leishmaniasis had been found in humans, domestic and wild animals were tested in search of reservoir species. All horses, mules (Equus equus and E. equus x Equus asinus) and dogs (Canis familiaris) were clinically examined, and indirect immunofluorescence test was also performed on serum samples from dogs.

Wild animals were trapped with metallic (Tender and Manufrance) traps, observed for 2 to 3 months and then killed under general anaesthesia. Smears and cultures of the skin, liver, spleen and bone marrow were examined for Leishmania spp.

Results

Human cutaneous leishmaniasis

During the study period 14 cases (8 males, 6 females) of cutaneous leishmaniasis were diagnosed (8 in 1987, 4 in 1988 and 2 in 1989). No cases of mucocutaneous or visceral leishmaniasis were detected. The annual prevalence of the disease was 3.81%

in 1987, 1.9% in 1988 and 0.95% in 1989. Table 1 summarizes the distribution of patients by age group and sex.

Table 1: Distribution of patients by age and sex

Age Group (years)	Male	Female	Total
0 - 14	0	0	0
5 - 14	0	4	4
15 - 44	5	1	6
45 +	3	1	4
TOTAL	8	6	14

Of the male patients, 7 were farmers and 1 was a public employee; of the female patients, 2 were housewives, 3 were schoolgirls and one was a baby girl. The patients had from 1 to 5 lesions: 9 had 1 lesion, and 5 had 2 to 5 lesions. Among all patients we observed 26 lesions distributed as follows: trunk 10, arms 9, legs 6, face 1. Most lesions were ulcerous; of 20 such lesions, 11 were crusted, 6 wet, 2 verrucous and 1 purulent. There were 4 nodular and 2 plaque-like lesions. At the moment of examination, the lesions had been present for periods ranging from 15 weeks to 1 year.

All patients were treated with meglumine antimoniate (Glucantime) at doses of 10-20 mg Sb 5/kg/day until all lesions had resolved. Mean duration of treatment was 20 days, with a range of 10 to 24 days.

The elevation of the site of residence of all patients was between 800 and 1300 m.a.s.l., most patients (66%) living at approximately 1000 m.a.s.l.

Montenegro tests were performed in 156 schoolchildren between 6 and 12 years of age. Of this sample, 101 children (65%) were positive (induration $\geq$ 5 mm.).

Identification of the parasite

The strains of *Leishmania* spp., isolated from patients were identified with monoclonal antibodies (mAbs) and isozyme electrophoresis at the CIDEIM Foundation in Cali, Colombia, with monoclonal antibodies provided by Dr. C. McMahon Pratt. All strains reacted at a dilution of 1:1000 with mAbs B16, B18, B21 and B12, and were therefore typified as *Leishmania braziliensis*.

Thirteen enzyme standards were used in electrophoresis studies (MPI, G6PD, NH, 6PGD, ASAT, ALAT, PEP-D, ACP, PGM, PEP-1, ES, GPI and SOD). All strains were identified as L. braziliensis differing from variant of reference strain M2903 by enzymes MPI (Mannose phosphate isomerase) and PEP-1 (aminopeptidase 1), and corresponding to Cali zymodene 11.

Phlebotomine sandfly fauna

Of the 10 species of phlebotomine sandfly captured and identified, 9 belonged to the genus Lutzomyia (Lu), and one to the genus Warileya (Wa.). The flies were distributed through a range of elevations from 800 to 2000 m.a.s.l., as shown in Table 2 and Figure 2, and were active throughout the year.

Table 2: Distribution of species of phlebotomine sandfly captured in Montebello, Colombia at different elevations

SPECIES	ALTITUDE (m.a.s.l.)
<u>Lu. rosabali</u> (Fairchild & Hertig, 1958)	800
<u>Lu. gomezi</u> (Nitzulescu, 1931)	800-1300
<u>Lu. venezuelensis</u> (Flock & Abonnenc, 1948)	800-1300
<u>Lu. lichyi</u> (Flock & Abonnenc, 1950)	800-1300
<u>Wa. rotundipennis</u> (Fairchild & Hertig, 1951)	800-1300
<u>Lu. hartmanni</u> (Fairchild & Hertig, 1957)	800-1600
<u>Lu. dubitans</u> (Sherlock, 1962)	800-1800
<u>Lu. nuneztovari</u> (Ortiz, 1954)	1000-1850
<u>Lu. youngi</u> (Feliciangeli & Murillo, 1987)	1000-1850
<u>Lu. moralesi</u> (Young, 1979)	2000

The most abundant species was Lu. gomezi (715), followed by Lu. venezuelensis (14%) and Lu. youngi (8.5%) (Table 3). Lu. gomezi was the species most frequently captured in the act of biting humans (92%), followed by Lu. youngi (6%).

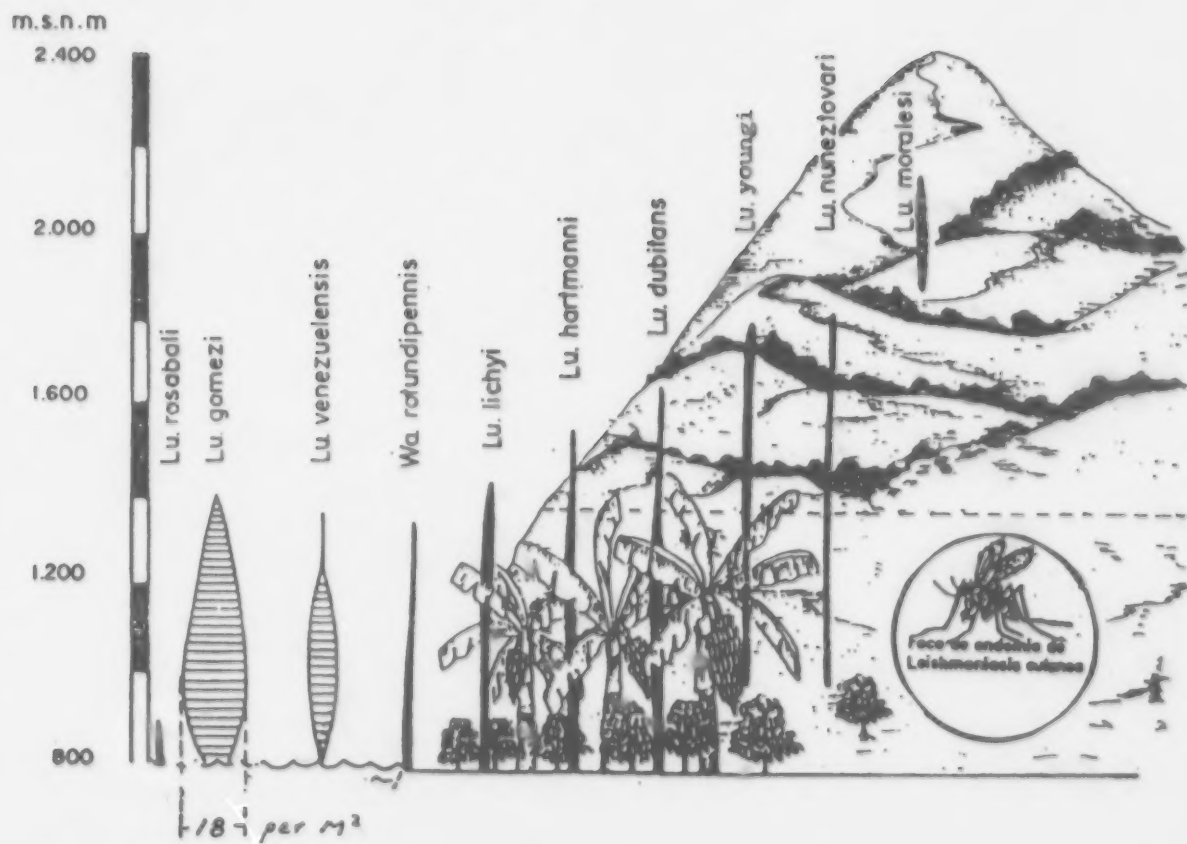


Figure 2. : Altitudinal distribution and density of phlebotomine sandflies in Montebello, COLOMBIA

Table 3: Percentage capture of phlebotomine sandflies with paper traps soaked with castor oil

<u>Lu. gomezi</u>	71.3%	<u>Lu. dibtans</u>	0.8%
<u>Lu. venezuelensis</u>	14.1%	<u>Lu. nuneztovari</u>	0.6%
<u>Lu. youngi</u>	8.5%	<u>Wa. rotundipennis</u>	0.56%
<u>Lu. moralesi</u>	1.85%	<u>Lu. lichyi</u>	0.4%
<u>Lu. hartmanni</u>	1.7%	<u>Lu. rosabali</u>	0.12%

In all species, density per square meter varied at different elevations throughout the year. Figures, 3, 4 and 5 illustrate the density of Lu. gomezi at 800, 1000 and 1300 m.a.s.l. respectively. Density was greatest at 1000 m.a.s.l., with a peak density of 98 individuals per m², and an average of 15.9 individuals per m² during the study period.

Density of all species of phlebotomine sandflies was greatest during rainy periods. Figure 4 shows the changes in monthly density of Lu. gomezi at 1000 m.a.s.l. with rainfall. Figures 6 and 7 shows the circadian activity of Lu. gomezi and Lu. youngi.

On the basis of its geographical distribution coinciding with the zone of transmission, its high population density, anthropophilic behaviour and domestic biting habits, in addition to its documented capacity to act as a vector (10), we suspected that Lu. gomezi is the vector species in this focus of cutaneous leishmaniasis.

Domestic and wild animals

In total, we examined 34 dogs and 20 horses and mules, which made up 100% of the canine and equine populations in the study area. Indirect immunofluorescence studies to detect Leishmaniasis were performed on serum samples from all dogs. In no animal did we find lesions compatible with leishmaniasis, and antibody titres were likewise negative.

The following wild animals were captured in peridomestic areas near dwellings whose occupants had leishmaniasis: 10 Didelphis marsupialis, 8 Rattus rattus, 17 Mus musculus and 1 Sciurus granatiensis. None of these animals showed clinical signs of lesions compatible with leishmaniasis, and parasitological analyses failed to reveal the presence of Leishmania in any of them.

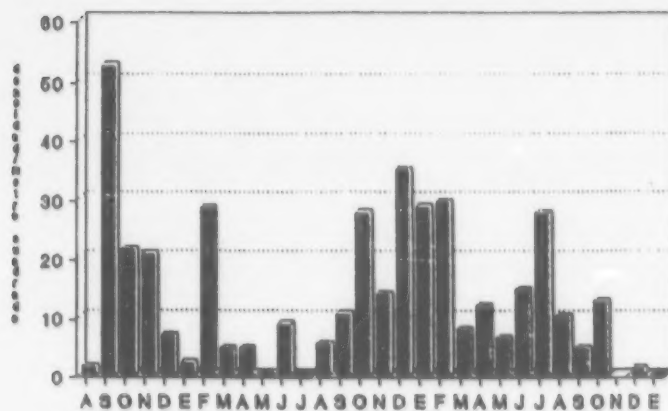


Figure 3. : Density of *Lu. gomezi*/m² at 800 m.a.s.l.

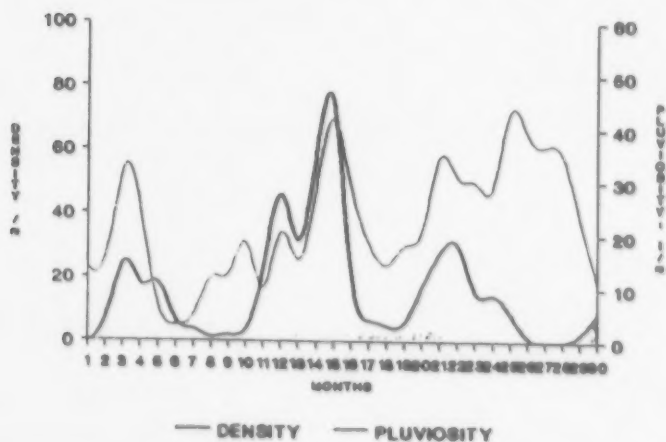


Figure 4. : Pluviosity and *Lu. gomezi* at 1.000 m.a.s.l.

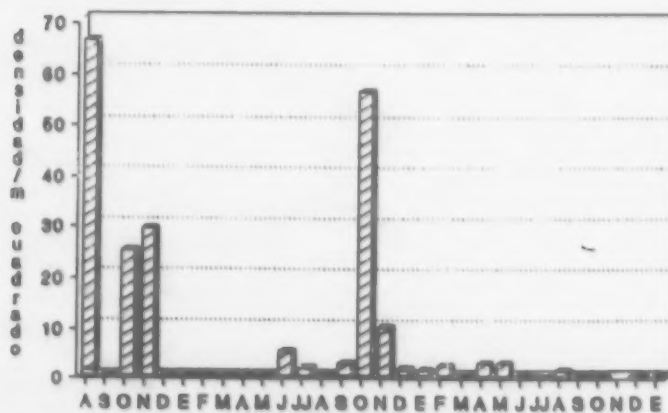


Figure 5. : Density of *Lu. gomezi*/m² at 1.300 m.a.s.l.

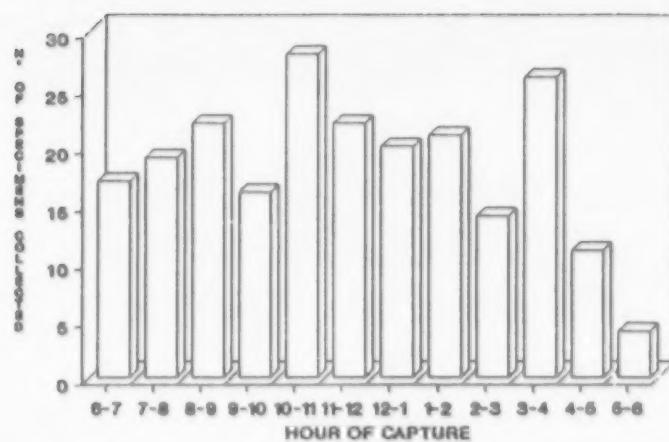


Figure 6 : *Lu. gomezi* activity

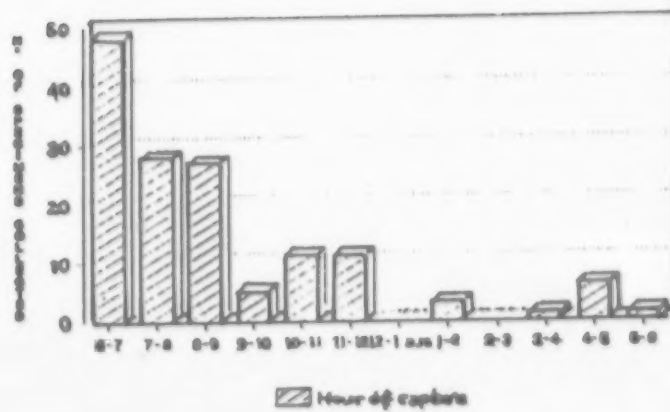


Figure 7 : *Lu. youngi* activity

Discussion

Thanks to active searching and early treatment of patients the prevalence of cutaneous leishmaniasis in the Montebello region decreased from 3.81% in 1987 to 0.95% in 1989. However, the 1989 figure was still higher than the corresponding prevalence in regions where the disease was known to be most common: 0.75% and 0.76% respectively in the Rio Magdalena Valley and Bajo Cauca Valley regions.

All patients diagnosed in this study lived at elevations ranging from 800 to 1300 m.a.s.l., i.e., the same range as that where Lu. gomezi was found to be most common. Moreover, most patients lived at elevations where the population densities of this species was highest (approximately 1000 m.a.s.l.).

Given the probable domestic nature of transmission, the disease affected all members of the family regardless of age or sex. The type of lesions and their anatomical distribution matched those classically described in association with American cutaneous leishmaniasis, however, it should be pointed out that although the disease is caused by a variant of L. braziliensis, the mucosa were not affected in any of the patients we studied, including those who had been ill several years before the study was performed.

The high rate of positive results on the Montenegro test among schoolchildren (65%) reveals that contact between humans and the vector occurred in domestic and peridomestic areas, and that infection occurs within the first few years of life at an incidence of around 10% per year. Much infection must be cryptic.

The elevation distribution of the probable vector species limits the focus to areas between 800 and 1300 m.a.s.l., and facilitates the rational planning of measures to eradicate the disease, which should be concentrated at these elevations although phlebotomine sandflies are found at elevations of up to 2000 m.a.s.l.

The presence of 10 species of phlebotomine sandflies in the study region reflects the variety of phlebotomine fauna in Colombia, where 127 different species have been reported to date. The present study represent the first report of Lu. youngi in Colombia.

The endemic presence and epidemic outbreaks of cutaneous leishmaniasis in Montebello, where all members of the population are at risk of infection, led us to suspect the presence of a domestic animal reservoir. The absence of anti-Leishmania antibodies in dogs, and of lesions compatible with cutaneous leishmaniasis in dogs, horses and mules, bolsters the notion that humans themselves act as a reservoir during an epidemic outbreak (11). This would give rise to a peridomestic cycle which would maintain the endemic in the study region, with Lu. gomezi serving as the vector and as yet unidentified organism acting as a reservoir. Epidemics may occur by a domestic, anthroponotic cycle set in motion when the number of active cutaneous lesions in the focus rises sufficiently; in epidemics the vector species is also Lu. gomezi.

The decrease in prevalence during the three year study period may be explained by early diagnosis and suitable treatment, which prevented the spread of the disease and transmission by new vectors.

Conclusions

The findings of the present ecöepidemiological study allowed us to determine the epidemiological risk of contracting cutaneous leishmaniasis within the region limits of Montebello, Colombia.

The risk zone, where most cases of cutaneous leishmaniasis were detected, is situated between 800 and 1300 m.a.s.l., at elevations where Lu. gomezi is distributed. Risk is highest at an elevation of 1000 m.a.s.l., where the density of this species of phlebotomine sandfly is greatest. The period of greatest risk is towards the end of the rainy season, when the Lu. gomezi population increases. The human population at risk comprises all persons living at elevations between 800 and 1300 m.a.s.l., regardless of age, sex, and occupation. The risk of contracting cutaneous leishmaniasis is greatest in domestic areas, where contact between humans and the vector is most intense, especially after 1800 hours and throughout the night.

The determination of epidemiological risk allows us to formulate control measures, which should be based on two main aspects. The first aspect deals with the control of the vectors. This requires the evaluation of measures aimed at preventing Lu. gomezi bites in the population; such measures should be implemented at elevations between 800 and 1300 m.a.s.l., in domestic and peridomestic areas, and should be especially concerted at the end of the rainy season. All persons should avoid being bitten, although efforts should be concentrated on protecting children. Among the measures worthy of consideration are: the planting of trees and shrubs containing natural pesticides, the domestic and peridomestic application of long-acting pesticides, the use of pesticide-impregnated mosquito nets and the application of insect repellents before bedtime.

The second aspect involves control of human activities. Since humans seem to act as reservoirs for the parasite, the disease should be diagnosed and treated promptly, to spare the patient as much suffering as possible, alleviate morbidity, and to reduce the spread of the disease and the appearance of new epidemics.

These suggestions involve intervention at three different levels:

- 1) Community education about leishmaniasis, its clinical appearance, etiology, mode of transmission, diagnosis and treatment, so that inhabitants will recognize the disease, seek medical treatment promptly, and participate in control measures.

- 2) Continuing education courses for private and public health service doctors, to enable them to recognize cutaneous leishmaniasis despite its clinical polymorphism, and to teach them to collect and forward samples correctly for parasitological analysis, the effectiveness of which depends to a large extent on the sensitivity of the test used.
- 3) Training rural health officers to actively search for cases, provide correct treatment, and cooperate with community health education programs. These officers in turn would maintain community-wide motivation to actively participate in campaigns aimed at controlling the vector.

Acknowledgements

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Geographic Distribution and Ecological Risk Factors Associated with the Transmission of Cutaneous Leishmaniasis in Jordan

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Introduction

For several decades cutaneous leishmaniasis (CL) has been known to occur in most of the Near East area, including Jordan. However, recently the disease has become an important public health problem in Jordan. The first documented Jordanian cases occurred in the seventies when 524 patients were seen at the King Hussein Medical Center during the period from 1973 to 1978. Of those cases, 259 (49%) came from the Jordan Valley (Oumeish, Saliba and Allawi 1982).

In the mid 1970s, Jordan experienced an economic boom. Land reclamation and agricultural development and modernization were intensified and urban and semi-urban areas extended into previously arid land leading to the settling of many bedouin tribes, primarily in these reclaimed and developed areas.

The magnitude of the problem of CL in Jordan is not yet determined with certainty. It is worthwhile to recall that in most of the developing countries, reporting in general is incomplete and rather inaccurate. Reporting of infectious and parasitic diseases is the first to suffer.

Our research project aims to address many of the unanswered questions surrounding the problem of CL in Jordan. Most important are the actual prevalence and annual incidence, the socio-economic, behavioral and ecological determinants of disease spread and geographic propagation. However, because the project is still in its early phase of implementation, we shall present in this paper the major objectives of the research proposal as well as the methodology to be employed. Preliminary findings obtained recently will also be highlighted.

General objective

The general objective or purpose of the project is to study the epidemiology and the physical and human ecology of CL so that appropriate preventive and control measures can be suggested.

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The specific objectives of the project are as follows:

1. To determine the current magnitude of the problem (the period prevalence of CL in Jordan 1980-1989).
2. To identify the geographic distribution of the disease (currently endemic areas).
3. To identify community and ecological factors involved in disease dissemination.
4. To determine the incidence of CL in Jordan for the year 1992.

The expected outcome of the project is: (i) identification of routes of recent spread of CL; (ii) identification of currently disease-free areas where a future spread of CL may be probable (areas at risk); and (iii) identification of intervention targets that may be effective in prevention, control and possible eradication of CL in Jordan.

Study Design and Methodology

In order to determine the current magnitude of the problem (the period prevalence of CL in Jordan 1980-1989) a case of CL should satisfy the following criteria: clinical diagnosis confirmed by positive laboratory tests (i.e. a positive smear and/or a positive culture. In some confirmed cases other laboratory studies may be required to ascertain the species of the parasites.

Two types of patients may be seen. The first are those with both clinical and laboratory confirmation and the second with clinical diagnosis only where no laboratory tests were ever made or are available. These two groups will be treated separately when results are finally analyzed. Cases shall be collected through the attached questionnaire.

Case finding shall be carried out by personal contact with institutions and persons all over Jordan. These are: a) The Royal Medical Services; b) The Ministry of Health with its hospitals, primary health care centers, school health services and laboratory services; c) The private sector: general practitioners, dermatologists, and private hospitals; d) The United Nations Relief and Work Agency (UNRWA) for Palestinian Refugees and displaced persons; e) voluntary agencies providing health services in endemic areas (Save the Children Fund, etc.).

Initially it may be sufficient to identify the geographic location of the patient which is of prime importance to determine areas of disease concentration (endemic areas). During initial contacts, project staff shall endeavour to establish a communication network to be utilized in the following phases of the study. Attempts shall be made by project staff to locate and interview each and every case detected, especially those

diagnosed in the late 1980s. When located, an individual questionnaire shall be completed. In this step the high mobility of the population will be taken into consideration.

The total number of cases identified for 1980-1989 will determine the magnitude or the period prevalence. This prevalence may be calculated according to the following formula:

$$\text{Period Prevalence} = \frac{\text{Number of existing cases during a period}}{\text{Average population}} \times 100,000$$

In order to identify the geographic distribution of CL (currently endemic areas) cases identified by completed individual questionnaires (specific objective 1) shall be grouped by geographic areas, according to the address given by the patient. This mapping by districts, the smaller administrative divisions, will indicate the areas of high disease concentration or currently endemic areas. A map of Jordan will illustrate this distribution.

In this context, it is important to note that an apparent increase in disease prevalence in a given area may not necessarily be the result of a real increase in the occurrence of CL but merely the effect of an increase of available health services or in the awareness of medical and health staff or both.

To implement the objective of identifying the community and ecological factors involved in disease dissemination a special instrument is needed. It is intended to explore the following topics:

- a. Community factors or social ecology: land reclamation projects, human migration and pattern of population movements, old and new settlements, place of origin of settlers, patterns of relationship of humans with domestic and wild animals.
- b. Physical ecology: including physical, geographic, agricultural and vegetation characteristics of the area.
- c. Animal ecology: areas from which cases of CL are reported will be visited and surveyed. The survey will include:
 - Signs for the presence of rodents (i.e. burrows) in zones extending up to 2 km around the human settlement.
 - Characteristics of burrows, number of openings, diameter of opening, orientation, distance between burrow systems, etc.
 - Species of vegetation present close to burrows. Evidence of feeding on plant species.
 - Chemical and physical characteristics of the soil where burrows are found (e.g. soil composition, sizes of particles, salinity, etc.).

- Climatic characteristics including temperature, rainfall and humidity.
- Species of rodents present. For this purpose, flooding of burrows, trapping and digging of burrows will be employed.
- Isolation of Leishmania parasite from collected animals will be attempted. Smears and cultures from skin lesions (ears), livers and spleens will be carried out. Schneider's medium or equivalent will be used for culturing the parasite. The isolated parasite will be typed enzymatically through the use of cellulose acetate electrophoresis as described by Saliba et al. 1988.

Attempts to collect sandflies from dwellings, animal barns and rodent burrows in the areas from which cases are reported will be made. Caster oil coated papers and light traps will be employed and the collected insects will be identified with the help of Dr. Kamhawi at Yarmouk University and WHO Collaborating Centers.

Information needed to complete the questionnaire shall be collected by field or site observation, interviews with administrative, health and social personnel and community activists and by reviewing pertinent reports and statistical data.

To complete the investigation two study districts from endemic areas will be selected as follows. Once the cases diagnosed in the period 1980-1989 are determined their addresses shall be plotted by districts and the districts stratified by the order of cases detected from the lowest to the highest. This frequency shall be divided into three groups: the highest, the middle and the lowest. The lowest frequency districts are those where less than twenty cases were detected, the middle from 20 to 50 and the highest with 50 and above. Two districts from the middle group shall be selected as the study districts.

Two disease-free districts similar to the two study districts in human and physical ecology shall be selected as controls and subjected to the same human, animal and physical ecological studies. Results will be compared to those obtained from the two study districts and analyzed accordingly.

It is suspected that animal-human transmission of CL may be the predominant pattern, but the human-human transmission should not be ruled out a priori.

Although L. major has been identified as the agent, L. tropica should not be definitely excluded, especially in the center of old cities or settlements. Laboratory studies shall be performed to assess the type of the agent involved in rural and urban areas.

To determine the incidence rate of CL in Jordan for the year 1992, individual questionnaires should be completed for all newly discovered cases. Diagnosing facilities or physicians contacted during the previous phase will be requested to fill individual questionnaires distributed to them.

If it is felt that this may not provide the necessary completeness and accuracy of information, another approach could be used: one person in the facility (a nurse or an employee in the administration) and the sole physician will be asked to keep a list of patients by name and address. These designated persons will be contacted personally or by telephone by project staff at regular intervals. A staff member will then contact the patient and complete the individual and area questionnaires. Collection of data in this phase should be very flexible and adaptable to each situation. The most important goal should be to identify and locate each and every patient diagnosed. The project team will be available to complete the task there after.

If the newly detected patient is clinically diagnosed, a smear and culture will be prepared either by the physician or the project team. All smears shall be examined or re-examined at the University of Jordan. All bio-chemical studies will be performed at the University of Jordan laboratory.

With this information network it is expected that the number of new cases missed shall be reduced to a minimum. Between the physicians, the laboratories, the facilities (hospitals and health centers) and the school teams all newly detected cases should be known. The project follow up and periodic checks shall provide a unique and efficient reporting channel. By the end of the year 1992 a complete list of newly diagnosed cases shall be available.

Incidence can be calculated as a rate per 100,000 midyear population. The formula used is:

$$\text{Incidence rate} = \frac{\text{number of new cases/year}}{\text{population at midyear}} \times 100,000$$

It is expected that through this investigation it will be possible to identify areas which are currently disease free and where CL may be expected to appear in the future (area at risk).

Disease-free areas where significant community and ecological risk factors are found could be suspected as areas at risk where CL may appear if no preventive measures are taken. A map of Jordan shall be used to illustrate these findings.

Data collected through individual and area instruments shall be transferred to the computer as soon as each questionnaire is checked, verified and deemed to be final, complete and accurate. Computer analysis may be started on sample numbers of the questionnaires, but the final results will not be released until after data collection is finished.

Data analysis will investigate the variables explored in the questionnaires, their intrinsic importance and their relationship. Correlation of community and ecological factors, and their variations with disease causation will be emphasized.

Among important variables to be tested and correlated to disease causation are: age, sex, education, occupation, location (urban-central or suburbs-rural), altitude, season, development projects and migration, household income, dwelling structure and sanitary conditions, crowding, human relation to domestic animals, attitudes, beliefs, values and practices regarding: health and disease, CL, wild animals and rodents, vector, sanitation, personal hygiene, health facilities, health education and health messages.

Several statistical methods will be used: tabulation, cross tabulation, significance tests, correlation, multiple regression, and other statistical procedures whenever needed.

Models, computerized or others, may be constructed to clarify the process of disease occurrence. A qualified statistician from the University of Jordan will help to design instruments and analyze data.

The final report of the project will attempt to explain in detail the epidemiology of CL in Jordan, with emphasis on risk factors, socio-economic, community, behavioral and ecological factors involved. Finally, a special section will be devoted to suggesting a broad set of interventions, geared toward enhancing or establishing preventive measures for complete control.

Results of this project and suggested intervention measures shall be disseminated locally, regionally and internationally through:

1. Seminars and workshops for target groups such as personnel in health, social, voluntary, community action and administrative fields who are decision-makers in the process of prevention, detection, diagnosis, treatment and control of CL. Participants will be selected from Jordan and the Arab region. International facilitators may be invited. Topics will center on the project objectives, methodology and results, and on recommendations for control. It is anticipated that two such seminars will be held; one in year II and one in year III of the project.
2. Papers based on the project will be published in local, regional and international medical and health journals.
3. Papers will be presented to local, regional and international conferences and seminars.
4. Contact with regional and international workers in the field of CL through attendance at conferences and other relevant scientific gatherings.

Preliminary Results

Data obtained on cases diagnosed between 1980 and 1989 is being compiled. Although the figures are not yet final, they clearly indicate that the Jordan Valley, specifically the strip of land which lies between Deir Alla and Sweimeh (30 km long) and

which extends from 3-5 km to the East of the River Jordan, is the major endemic area in the country. This strip represents the upper limit of the Sudanian region but it also incorporates elements of the Irano-Turanian region (Al-Eisawi 1985). Many succulent plants are present including Atriplex, Seidlitzia, Suaeda and others. Burrows of rodents which were located were predominantly associated with Atriplex.

The data available show that outbreaks have occurred in areas considered free of the disease earlier. Two of these areas are worth noting; Al-Mowaqqar and Al-Hasa. Between 1982 and 1983, more than 193 cases were reported from Al-Mowaqqar area some 15-20 km to the East of Amman (Saliba et al. 1985). The area is Irano-Turanian with the succulent plant, Anabasis Syriaca, predominating (Amr and Saliba 1986). Colonies of the fat jird, Psammomys obesus were collected from the vicinity of the villages in the area and even from burrows located 50-100 metres from houses. The parasites isolated from humans and jirds were enzymatically identical to Leishmania major (Saliba et al. 1988).

In 1988-1989, more than 100 cases occurred in Al-Hasa village, some 165 km south of Amman, on the main highway to Ma'an and Aqaba. The village has expanded in the last few years due to the establishment of phosphate mining facilities in the area. Again, Al-Hasa area is Irano-Turanian with A. syriaca predominating (Al-Eisawi 1985). Because the water used in washing phosphate flowed westward in the direction of the village, A. syriaca flourished east of the village. Colonies of P. obesus and Meriones libycus rodents were located few hundred meters to the east of the village and active burrows from which infected P. obesus were collected were less than 100-500 meters from houses or bedouin tents. So far, only P. obesus was found infected in Al-Hasa area. Furthermore, Phlebotomus papatasi sandflies have been collected from burrows as will be mentioned later.

Sporadic cases have recently been reported from areas thought to be free of CL. The areas include Malka area in the north, Al-Salt area, Al-Karak area and Al-Disi area in the south. The first two foci are peculiar since they are considered, phytoecogeographically, as Mediterranean and do not match the known habitats of ZCL encountered in other parts of Jordan. The nature of the disease in these new areas is being investigated.

The factors that have contributed to the appearance of cases in new areas as well as to the increase in the number of cases are also being investigated. We speculate that the following are important factors:

1. The considerable rehousing and construction of new houses in endemic areas recently.
2. Development of previously uninhabited areas where the reservoir and vector were present.

3. Environmental and ecological changes that favoured an increase in the population of both the animal reservoirs and the insect vector.
4. Awareness on the part of dermatologists and other practitioners.
5. Public and general concern over environmental impact of mass spraying with insecticides especially in the Jordan Valley in the course of the malaria eradication campaign.

To date, several rodent species have been collected from endemic areas either by flooding burrows or by trapping. However, only the fat jird, *P. obesus*, have been found infected with *Leishmania major* (Mon. 26 and 103). Of the 162 *P. obesus* collected, 36 were infected (Table 1). *Meiriones libycus*, *M. crassus* and *M. tristrami* have not been incriminated as reservoirs, so far (Table 2).

A summary of other results obtained thus far include:

1. Experimental infection of *M. Libycus* showed that laboratory-bred animals inoculated in the foot pad with 10^3 , 10^5 and 10^7 promastigotes of *L. major* developed thickened areas and the parasite was re-isolated within 3 to 6 weeks post inoculation (Table 3).
2. The following plant species or genera have been found associated with burrows of rodents: *Atriplex*, *Seidlitzia*, *Suaeda* and *Mesembryanthemum* (Jordan Valley), *Anabasis syriaca*, *Hammada* and *Noea* (Irano-Turanian strip) and *Haloxylon*, *Atriplex*, *Anabasis*, *Suaeda*, *Traganum* and *Zygophyllum* (Wadi Araba).
3. Attempts to collect sandflies from active burrows of rodents in two endemic areas (Al-Hasa and Central Jordan Valley) were carried out during the months of June to October 1991. The burrows were located at distance about 50-600 metres from houses or tents. CDC light traps modified to collect insects flying out of rodent burrows were used as well as castor oil coated papers. The only *Phlebotomus* species collected so far by the CDC traps was *Ph. papatasi*. In the Jordan Valley, 350 females were collected of which 200 were dissected as described by Rioux et al. 1986. On the other hand, 61 females were collected in Al-Hasa and all were dissected. Flagellated organisms were inoculated into semi-solid medium and into Schneider's *Drosophila* medium containing 30% fetal calf serum and antibiotics. However, the cultures were contaminated and the isolates were lost. Bacterial or fungal contamination of culture media inoculated with sandfly guts is not uncommon (WHO Expert Committee 1990).

Table 1: Number and Sex of Infected Psammomys obesus Collected from Different Parts of Jordan

<u>Area</u>	<u>Number of Animals</u>			
	<u>Females</u>		<u>Males</u>	
	<u>E</u>	<u>I</u>	<u>E</u>	<u>I</u>
Mowaqqar	30	7	31	3
Al-Hallabat	41	5	20	5
Um-Rasas	11	5	2	1
Qatrana	3	3	1	1
Khaldieh	10	0	2	0
Ma'an	4	0	-	-
Wadi Araba/Gharandal	2	1	-	-
Hasa	4	4	1	1
Total	105	25	57	11

Table 2: Species of Rodents Examined for Infection with Leishmania Amastigotes

<u>Species</u>	<u>NUMBER OF ANIMALS</u>	
	<u>Examined</u>	<u>Infected</u>
<u>Psammomys obesus</u>	162	36
<u>Meriones libycus</u>	65	--
<u>Meriones crassus</u>	12	--
<u>Meriones tristrami</u>	7	--
<u>Allactaga euphratica</u>	12	--
<u>Gerbillus dasyurus</u>	3	--
<u>Gerbillus nanus</u>	3	--

Table 3: Isolation of *Leishmania* parasites from experimentally-infected *Meriones libycus* rodents

Dose (promastigotes/20 μ l)	No. of positive rodents			
	3 weeks P.I.		6 weeks P.I.	
	Smear	Culture	Smear	Culture
10^3	0/8	0/8	7/8*	5/8
10^5	6/10	5/10	10/10	8/10
10^7	8/8	6/8	8/8	8/8

* The eighth rodent was positive in culture

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Epidemiological Studies on Andean Cutaneous Leishmaniasis and their Significance for Designing a Control Strategy

A. Llanos-Cuentas¹ and C. Davies²

Introduction

The Workshop "Research on Control Strategies for the Leishmaniasis" held in Ottawa, Canada in 1987, identified the priorities for research to aid control efforts. It recognized that each clinical-epidemiological form has unique characteristics, so that general recommendations are not feasible for control approaches. Four disease groups were considered separately. For one of them, New World cutaneous and mucocutaneous leishmaniasis, the major recommendations were: (1) the identification of high risk groups; (2) identification of the factors associated with transmission; (3) development of vaccines; and (4) pilot control studies.

Summary of the Status of Leishmaniasis in Perú

In Perú four *Leishmania* species have been described: *L.*(*V.*) *braziliensis*, *L.*(*V.*) *peruviana*, *L.*(*V.*) *guyanensis*, *L.* *mexicana* (Llanos-Cuentas 1990). Only the first two have epidemiological importance in Perú. *L.*(*V.*) *peruviana* causes Andean Cutaneous Leishmaniasis (Uta). It occurs in the Andean valleys along the Pacific slope and in some Inter-Andean valleys between 800 and 3,000 meters. Incidence also varies with latitude, and below 13° south there are no reported cases of Uta. In contrast with other subspecies of the *L.*(*V.*) *braziliensis* complex, *L.*(*V.*) *peruviana* is not transmitted in forested regions. In the endemic valleys of the western Andes, the vegetation in the uncultivated areas is usually low scrub. Uta patients always suffer cutaneous lesions, but mucous membranes can occasionally be involved via two processes: by extension of a contiguous lesion (Lumbreras 1985; Miranda 1988), or (very rarely) by metastasis (Llanos-Cuentas unpublished data).

L.(*V.*) *braziliensis* is the agent of cutaneous and mucocutaneous leishmaniasis (MCL) and it causes the majority of mucosal cases. It is present on the eastern slopes of the Andes, from 3,000 meters (in the Department of Huanuco) down to sea level and the Amazonian jungle.

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Leishmaniasis is a disease that is increasing in Peru; its incidence (as recorded by passive detection) has recently doubled from 9.1 cases/10⁵ in 1978 to 20.8/10⁵ in 1988 (Fig. 1). Both Uta and MCL have increased in this time (Fig. 2 and Fig. 3).

In some departments, such as Madre de Dios, Huanuco, Cusco, Ucayali and Ancash, incidence rates were above 70 cases/100,000 inhabitants in 1988; and in some areas, such as Ambo (Huanuco department), leishmaniasis has the same importance as respiratory disease, diarrheal disease or tuberculosis (Table 1).

Table 1. Principal causes of morbidity in Ambo from January to October, 1988

<u>Disease</u>	<u>No. of patients</u>
Leishmaniasis	364
Diarrheal disease	277
Respiratory disease	269
Tuberculosis	15

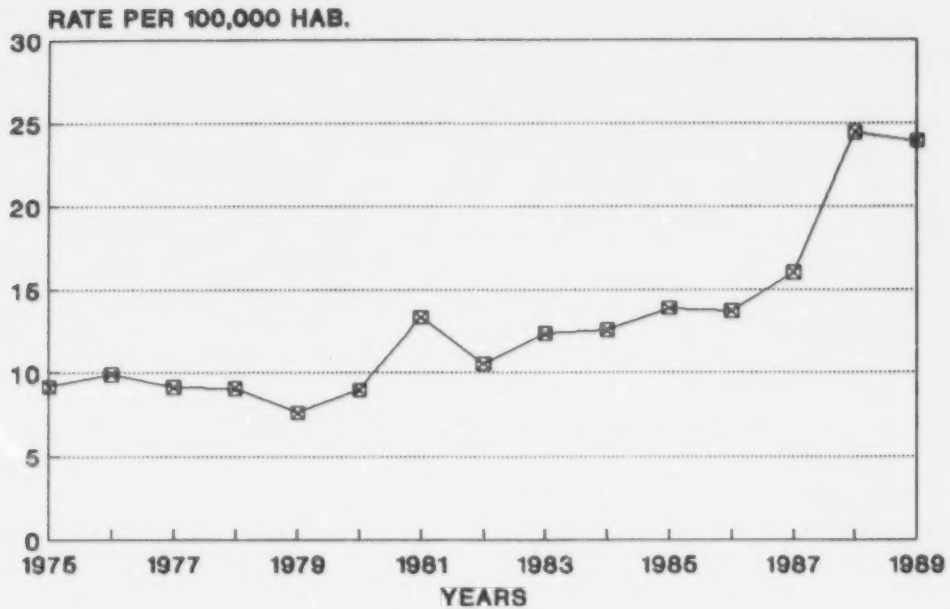
The Ministry of Health (MINSA) has estimated approximately 15,000 new cases of tegumentary leishmaniasis for 1992 (Chumbe 1991, manuscript document). There is no specific program for control in leishmaniasis in Peru, and the main measure taken by MINSA is the provision of drug supplies. Some operative units of MINSA have programmed spraying activities, but no quantitative analysis of their efficacy has been carried out.

Current Status of our Knowledge of the Epidemiology of Uta, and its Relevance for Control Strategies

In this meeting we would like to address the following questions: (a) Where and when does transmission of Uta occur? and (b) How does the epidemiology relate to the entomological parameters? These questions are potentially the most relevant for choosing an appropriate control strategy. We shall be referring to the results of recent and on-going studies that are being carried out in several endemic areas by the Working Group of Leishmaniasis at the Instituto de Medicina Tropical "Alexander von Humboldt"-UPCH and Centro de investigación en salud "Dr. Hugo Lumbreras"-INS-MINSA.

The identification of the vector species is one of the pre-requisites for any control program. Prior to our current studies, the evidence for distinguishing the vector status of the various potential vectors of Uta was largely circumstantial. At least nine Lutzomyia (Lu.) species (Diptera: Psychodidae: Phlebotominae) have been identified in sandfly collections made in endemic areas of Uta. The most important anthropophilic species are Lu. peruensis, Lu. verrucarum, and Lu. ayacuchensis.

FIGURE 1:
INCIDENCE OF LEISHMANIASIS IN PERU
1975 - 1989



Source: DTID/OGE

FIG. 2. NEW CASES OF UTA IN PERU
DURING THE PERIOD 1981 TO 1989

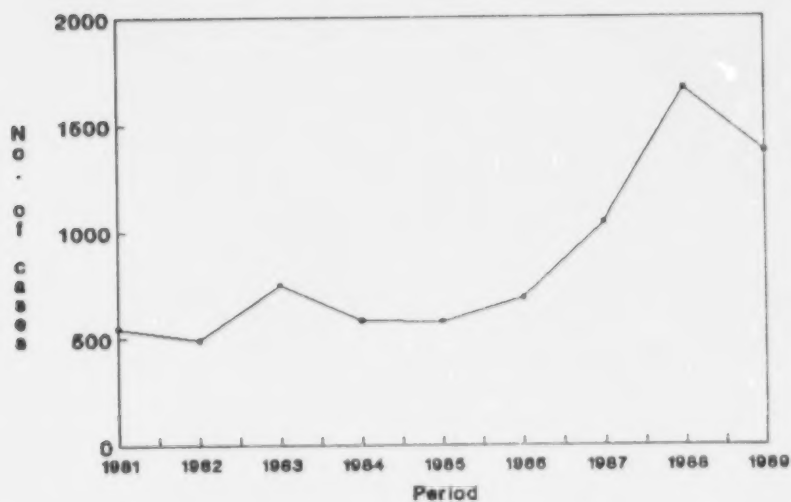
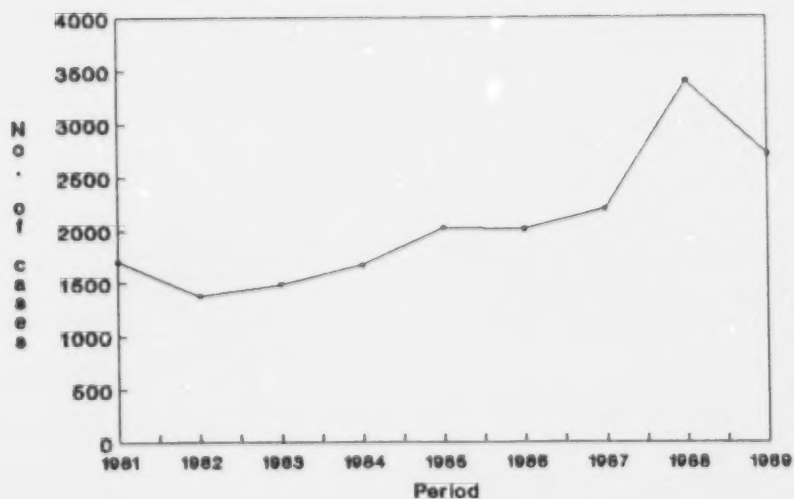


FIG 3. NEW CASES OF MUCOCUTANEOUS
LEISHMANIASIS IN PERU, PERIOD 1981-89



Following the early studies of Townsend (1913), Lu. verrucarum was incriminated as the vector of Bartonella bacilliformis, and transmission was shown to be closely associated with human dwellings. As Uta and bartonellosis are closely associated geographically, and frequently coexist in some areas, the vector and the place of transmission were assumed to be the same (Weiss 1943; Herrero 1951a; Herrero & Battistini 1957). No specific attempts were made to identify the vector of Uta until 1982 when Herrero (1982) induced Leishmania infections in hamsters (Mesocricetus auratus) inoculated with triturates of pooled field-caught Lu. peruensis. He concluded that this sandfly may be the natural vector of L.(V.) peruviana; he also suggested that Lu. peruensis has less domesticated habits than Lu. verrucarum (Herrero 1982). Later, Cruzado in La Libertad (1987) and Pérez in Ancash (Purísima valley) detected natural infections of Leishmania promastigotes in individually dissected Lu. peruensis (Pérez et al. 1991). The strain isolated by Pérez was identified by isoenzymes as L.(V.) peruviana. Recently, the group of Dr. Arevalo of IMTA-VH, using the polymerase chain reaction technique with a sequence of kinetoplast DNA specific for the L.(V.) braziliensis complex (López et al. 1991), have identified natural infections in two other species of sandflies: Lu. verrucarum and Lu. ayacuchensis (Arévalo, Cáceres & Pérez Personal communication). Lu. ayacuchensis is the likely vector in the endemic valleys of Ayacucho (and possibly in Piura), whilst in Western valleys along the Pacific slopes Lu. peruensis and Lu. verrucarum are the potential vectors. The relative importance of Lu. peruensis and Lu. verrucarum as vectors is hard to distinguish, as they are frequently sympatric and both show a close geographic correlation with the disease.

One approach is to relate altitudinal variation in the proportion of Lu. peruensis with variation in the incidence of Uta. In Figure 4, we can see clearly that the ratio of Lu. peruensis to Lu. verrucarum (collected in Shannon traps) increases with altitude, indicating that although the two species overlap, the mean altitude of the Lu. peruensis population is greater and correlates with an increase in the incidence of Uta with altitude (Fig. 5). Sandfly collections and prospective epidemiological surveys (by active search) were undertaken in Purísima valley from 1987 to 1989 (Phase I). The line of best fit was calculated by logistic regression. Thus, these results suggest that in Purísima valley transmission is mainly by Lu. peruensis, although a role for Lu. verrucarum cannot yet be discounted.

The second approach we are taking is to investigate temporal correlations between sandfly densities and monthly incidence rates. In Purísima valley, we correlated the monthly incidences in the whole valley with concurrent monthly variation in sandfly numbers in one highly endemic village (Huanchoc) estimated by three methods (CDC in houses, Shannon traps outside, and daytime captures from outdoor resting places by aspiration). The only relationship identified was between the number of Lu. peruensis caught inside houses and incidence one month later (Fig. 6). The correlation coefficient was 0.42 (just above the critical value (0.50) for significance ($p < 0.05$)). No correlations were observed for any other time shifts (from 0-6 months were tested) or for outdoor Lu. peruensis captures (by Shannon or aspiration); neither were any correlations apparent

FIGURE 4:
PERCENTAGE *L. PERUENSIS*
(Shannon traps in Purisima Valley)

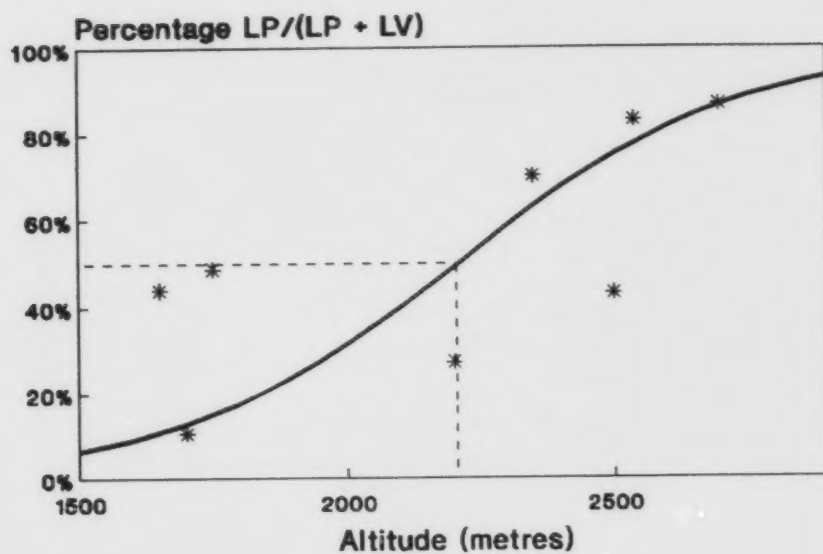
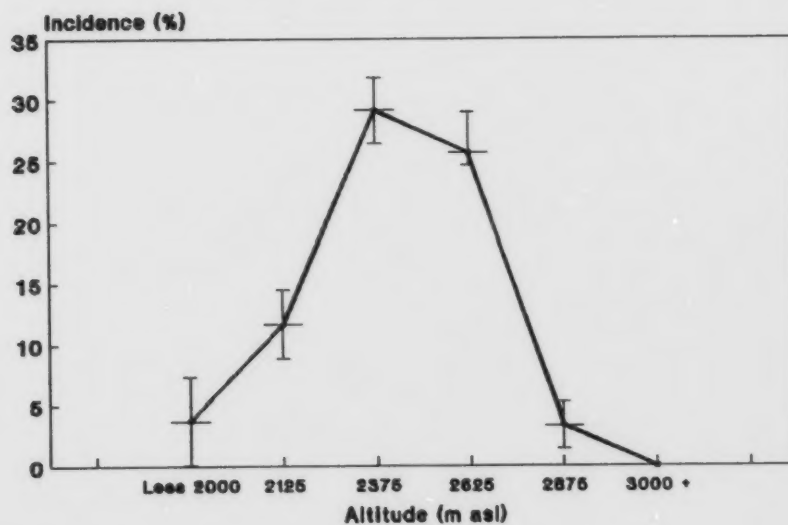
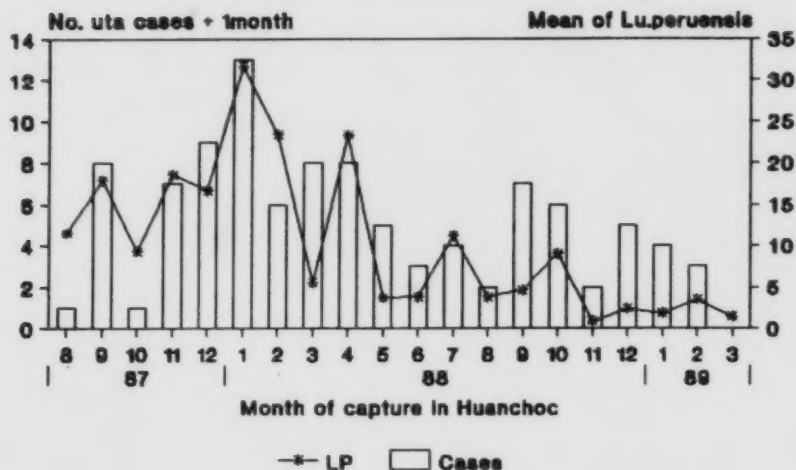


FIG 5. INCIDENCE BY ALTITUDE
(Purisima valley, Oct 86 - Mar 89)



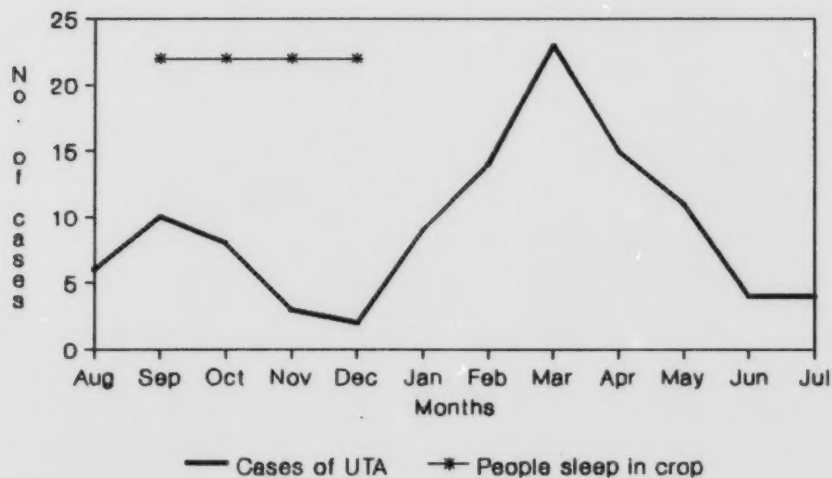
Incidence and confidence interval 95%

FIG 6. LU. PERUENSIS DENSITY AND MONTHLY DISTRIBUTION OF UTA CASES
(CDC inside houses; uta cases + 1 month)



Period August 87 to March 89

FIG.7. RELATIONSHIP BETWEEN DISTRIBUTION OF UTA CASES AND CROPS ACTIVITIES
(Purísima valley, August 87 to July 88)



with variation in the Lu. verrucarum population. These results suggest: (1) an incubation period of one month between sandfly bite and onset of lesion development; and (2) a key role for intra-domiciliary transmission by Lu. peruensis in the epidemiology of Uta in the Purísima valley.

Further on-going comparative studies of Lu. peruensis and Lu. verrucarum aim to: (1) relate incidence with sandfly densities in different endemic areas; (2) compare infection rates in the field; and (3) compare vectorial competence in laboratory transmission studies with colonised flies.

Additional observations in the Purísima valley indicate the possibility of extra-domiciliary rural transmission foci. The working habits of the population involve the displacement of all members of the family to their crop fields, where they remain for different periods (days), using provisional shelters. As these shelters are open, their occupants are very much exposed to sandfly bites. Sandfly captures were carried out at provisional shelters (both occupied and empty) in different locations of the valley, during the peak sandfly activity period (18:00 to 21:00 hours). The mean number of sandflies caught per night was from 20-31 in the occupied shelters, and less than 2 in the empty ones. Casual observation of (and conversation with) the population suggests that families remain in the crop fields (mainly corn) during the harvesting periods just some months before the peak of incidence in the valley (Fig. 7). A quantitative analysis of the time spent in these temporary shelters in relation to seasonal variation in incidence is currently being carried out.

It is vital to verify and define the different sites of transmission in order to account for the failure of past fumigation programs to eradicate the disease, and to design appropriate control strategies for the future.

Two further approaches are being taken to definitively identify the sites of transmission. Firstly, we are investigating the risk factors associated with recent transmission of Uta using a case control study. The exposure factors are the characteristics of the house, the peridomiciliary environmental characteristics, and the behavior patterns of the people in the endemic areas. Secondly, we are comparing monthly incidences and sandfly densities captured inside houses, in the peridomestic arena and within crops belonging to some 16 villages. The aim is to collect one year's base-line data, and then to manipulate the domiciliary sandfly populations (but not those in the crops) by house fumigation, and -- in a longitudinal active search study -- identify whether any reduction in incidence ensues.

The relative risk of intra-domiciliary infection versus the risk of infection associated with agricultural activities may well be age-related. A comparison of risk for different age groups could therefore provide clues to the relative importance of these two potential sites of transmission. Figures 8, 9, and 10 show the cumulative percentage of people infected in different age groups in four villages of Purísima valley (Ancash

FIGURE 8: Percentage of infected people in four villages of Purisima Valley

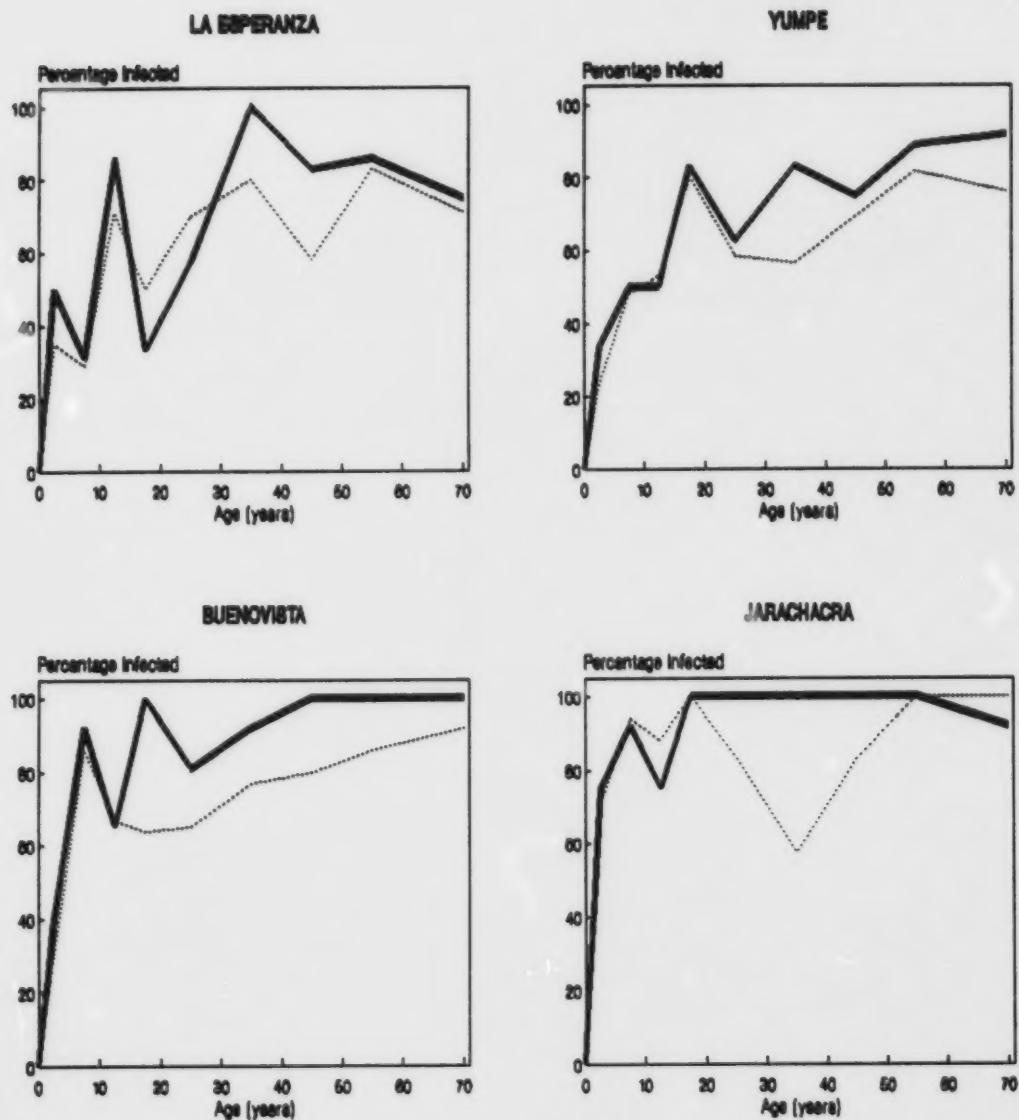


FIGURE 9: Percentage of infected people in four villages of Lurin Valley

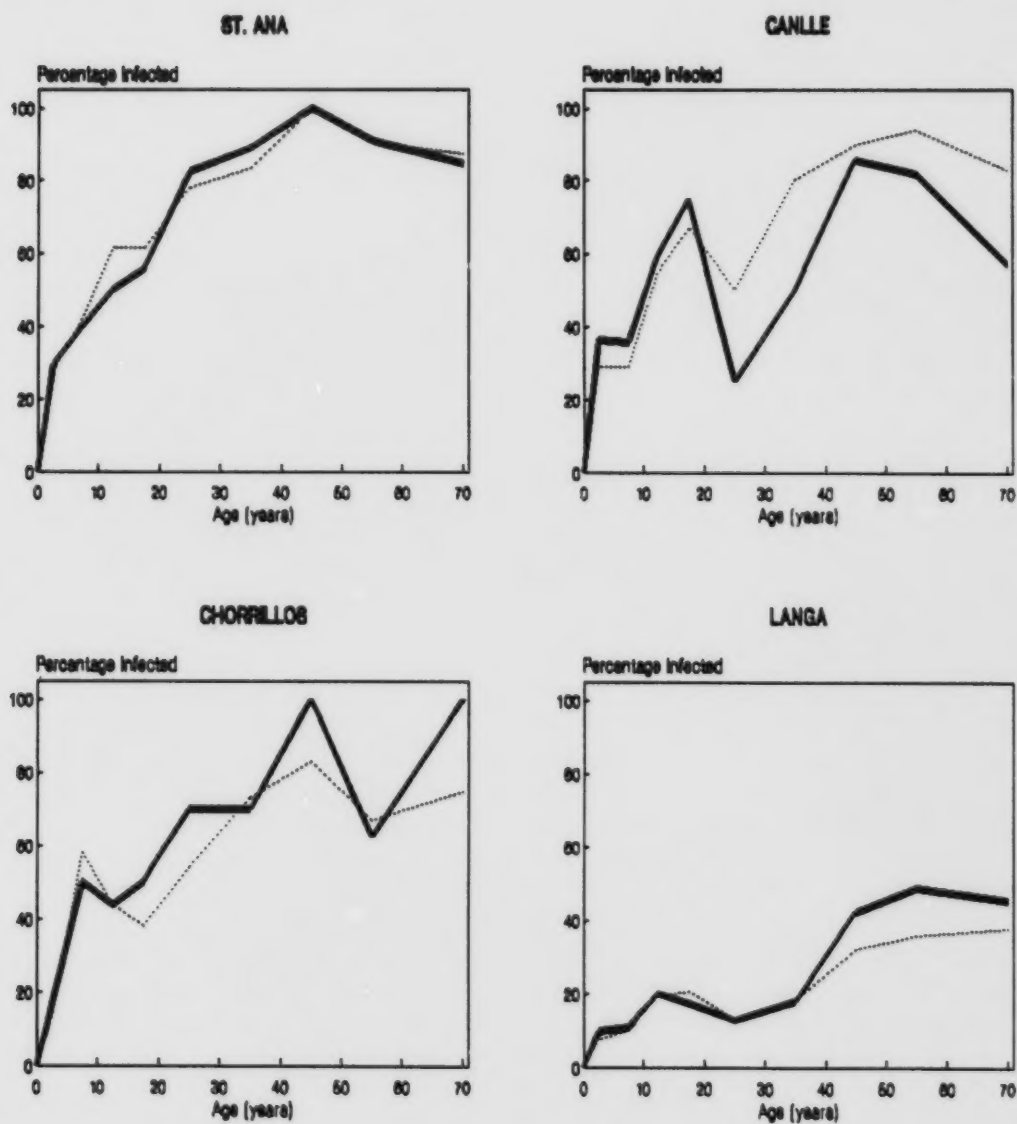
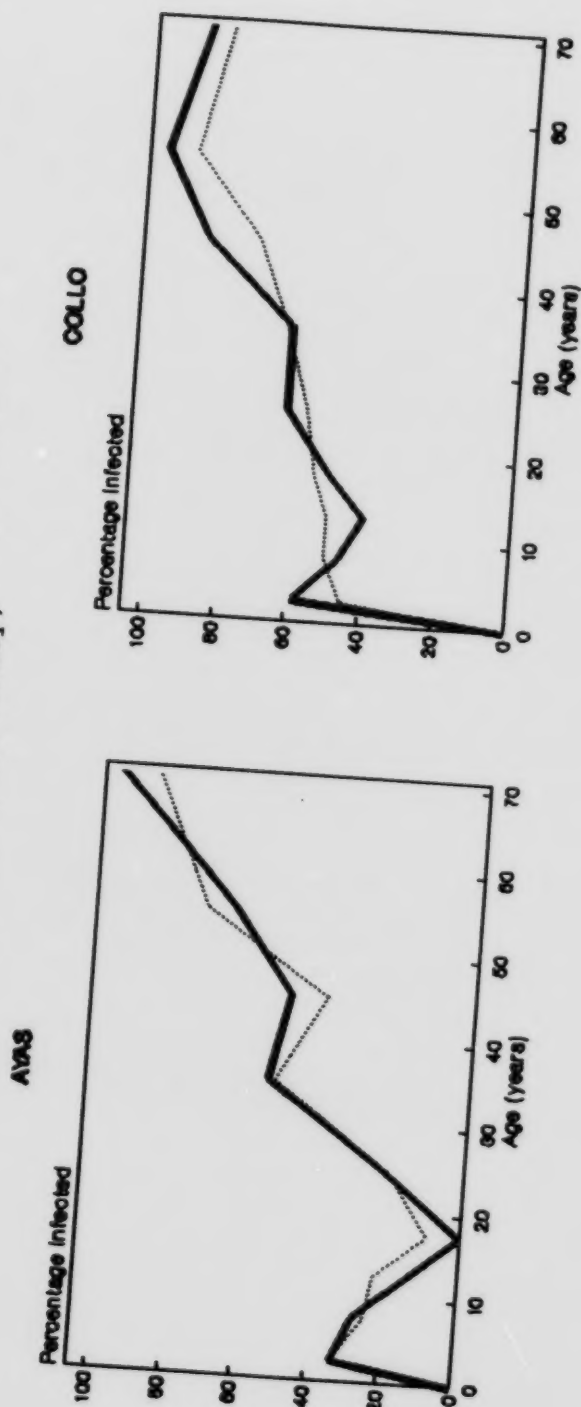


FIGURE 10: Percentage of infected people in two villages
(Rimac Valley and Canta Valley)



department), four in Lurín (Lima department) and two in Rimac Valley (Lima department). Present or past infections were identified in a cross-sectional house-to-house survey of the population by Montenegro test (solid line), as well as clinical signs -- characteristic scars or lesions (dotted line). Infections with *L. (V.) peruviana* lead to long-term protection against further infections, as illustrated by the relative rarity of second episodes of Uta in patients whose lesions have healed (i.e. with the characteristic scar). Thus, the villages with the highest infection rates (as measured by the cumulative prevalence for the whole population -- the percentage Montenegro positive), should have the lowest average age for cases. This can be visualised by comparing the graphs for Jarachacra, with the highest infection rate and a very high proportion of cases in children less than five, and Langa, with the lowest infection rate and the majority of cases in adults.

This is the pattern one would predict if the risk for susceptible (Montenegro negative) children and susceptible adults was the same. But the epidemiological patterns derived from the various villages in the study indicates significant deviations from any simple relationship between transmission rate and the proportion of cases in children. This can best be illustrated in Figure 11, which plots mean annual incidence (from 1989-1991) in susceptible children (under 15 years) for 16 endemic villages. Points near the x-axis represent villages where the infection rate in susceptible children is very much greater than that in susceptible adults; whilst points close to the y-axis represent the reverse. Points close to the dotted line represent sites with no change in incidence with age. The wide dispersion of points suggest the existence of different patterns of transmission, even for villages in the same valley. In some cases, such as Licahuasi, Canye and La Esperanza, the risk in susceptible children is eight times that in susceptible adults; whereas in Huatiacaya (Lurín) the risk to adults is four times greater than in children. Between these two extremes there are several intermediate patterns. Thus, these data suggest: (i) different patterns of age-related risk of exposure for people in different villages and/or different valleys; and/or (ii) differences in the immune status of the populations living in different endemic areas.

A similar pattern was generated from the results of phase I (epidemiological, clinical and parasitological characteristics of Uta) in the Purísima valley. There, the incidence risk in persons without signs of disease (scar and/or active lesions) fell rapidly with age (Fig. 12). The inference that asymptomatic infections (or at least those with no detectable scar) are frequent in this valley, and that immunity is developed by adults (probably as a result of multiple repeated infections), was not supported by our recent studies. Some 70% of the populations of the villages mentioned above (Fig. 8, 9 and 10) have undergone Montenegro skin tests. Ninety percent of all Montenegro positive inhabitants have detectable scars or lesions, and there is no significant difference in the percentage for children less than 15 years old (92%) and adults (89%). Thus, the drop in incidence rate with age for the unscarred population is apparently not generated by an increase in their immune status (at least as determined by the Montenegro test).

FIGURE 11:
Age-related incidence in susceptibles
Mean annual incidence in children(<15)
and adults in 16 villages: 1989-1991

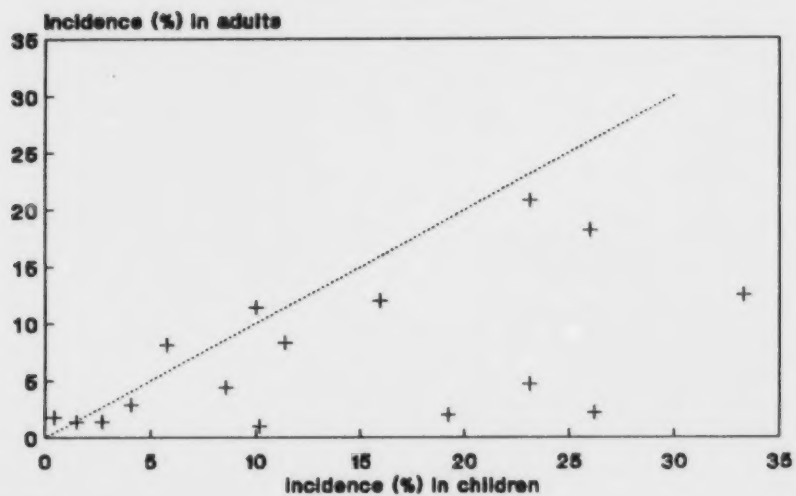
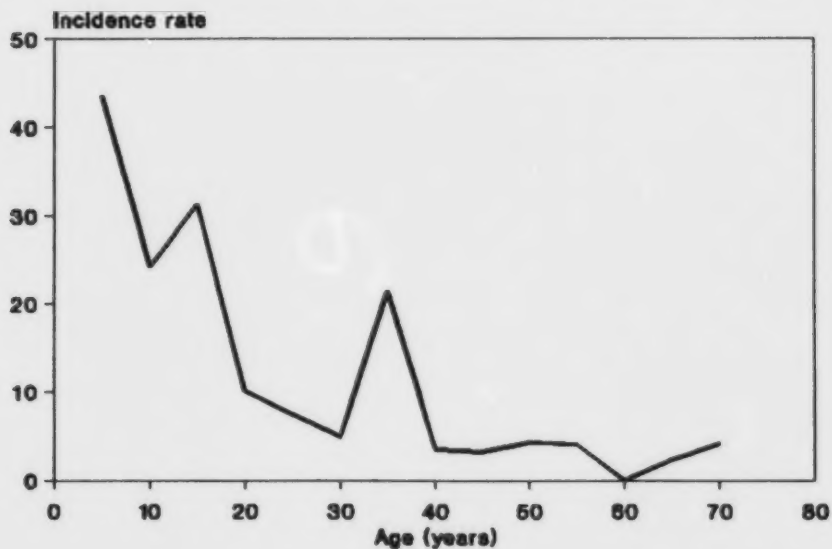


Fig. 11

FIG 12. INCIDENCE BY AGE
(Purísima valley, Oct 86 - Mar 89)



These results indicate that any decrease in incidence (in susceptibles) with age is probably due to a difference in risk (exposure) associated with age-related differences in human activity. In endemic villages or valleys where this is the case, there is a significant implication for disease control. Prevention of Uta for several years will undoubtedly increase the percentage of susceptible adults in the population, but if for some reason the control program was terminated, these susceptible adults would have a relatively low risk of infection (compared with what they would have experienced as children). Thus, the success of control programs in reducing the cumulative prevalence in the population should not be immediately reversed if they are suddenly terminated.

In Perú, during the last 40 years the control activities for leishmaniasis have focussed on DDT spraying campaigns. The field experiments carried out by Hertig (1942) and Herrer (1957) in the 1940s and 1950s demonstrated that DDT house spraying reduces the number of sandflies both inside the house and in resting places up to 30 meters from the sprayed house for up to one year. However, none of these studies carried out any controlled analysis of the effect of the spraying on disease incidence. Similarly, the experiences of MINSA were never evaluated. However, it is possible to examine the effect of DDT house spraying during the nationwide campaign to control malaria during the 1960s and first years of the 1970s. Leishmaniasis and malaria used to coexist in many of the Western Andean valleys. Figure 13 shows a dramatic drop in the incidence of Uta in Ancash department during the spraying campaign, followed by a rather slower resurgence once it stopped.

Similar patterns are being recognised in most of the villages that we are currently working in. Figure 14 illustrates the estimated mean annual incidence (in susceptibles) per decade calculated retrospectively from a cross-sectional survey of the populations of four villages in Lurin Valley, in which age, date of immigration (where relevant), disease status and age when infected for each inhabitant was recorded.

These results give a strong indication of the efficacy of DDT for reducing transmission related with human dwellings (inside and/or peridomestic). They also suggest a significant lag period after the spraying campaign was terminated before the infection rates returned to previous levels. However, unlike malaria, the spraying campaign clearly failed to eradicate leishmaniasis, possibly because the putative sylvatic transmission cycle of Uta was not affected by the reduction of sandfly populations associated with human habitations.

Nothing is known with any certainty concerning a sylvatic transmission cycle involving wild reservoir hosts. Although reservoirs have been studied in Perú since 1927 (Weiss unpublished data, referred in Herrer 1955), effort has concentrated on domestic animals (mainly dogs), possibly because dogs were the first animals found infected with *Leishmania* (amastigotes in smear) in the New World (Pedroso 1913), and because (jointly with bartonellosis) transmission was accepted as peridomestic (Herrer 1951a, 1957a; Lumbreras 1985).

FIGURE 13:
INCIDENCE RATE IN ANCASH (1959-1989)

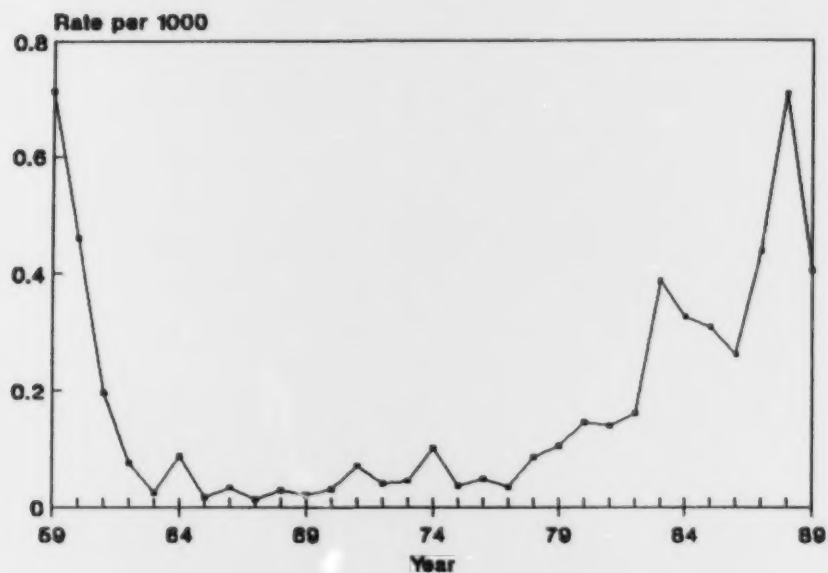
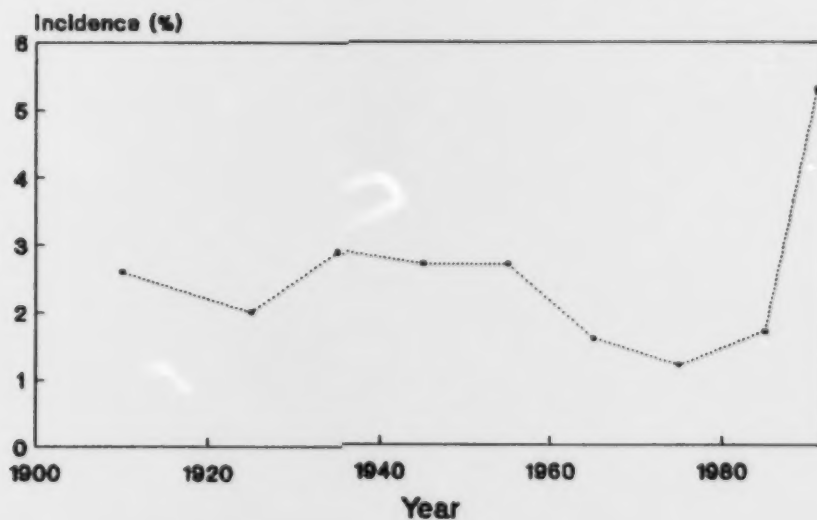


FIG.14. Change in incidence per decade
Mean annual incidence in unscarred
population in Lurin valley (LIMA)



Herrer & Battistini (1951), through experimental infection of dogs and foxes with promastigotes isolated from humans, demonstrated that although the majority of these animals did not develop ulcers, amastigotes could be detected in inconspicuous lesions. Based on this experience, Herrer carried out a field study (Herrer 1951b), in which he demonstrated amastigotes in stained skin smears (usually nasal) in 46 out of 165 dogs. No infections were found in 78 cats, 22 donkeys, 7 pigs or one horse. In a later study Herrer (1955) calculated a canine incidence of 29% in Huarochiri province, but his protocol for dog selection was not recorded. At that time a number of wild rodents and opossums were trapped and examined by the same method, but no parasites were demonstrated. Unfortunately, the descriptions of the methodology in Herrer's studies have frequently been incomplete making interpretation of his data awkward.

Recently, Llanos-Cuentas et al. (manuscript in preparation) studied the possible wild and domestic reservoirs in Purísima valley. The fauna was restricted: only three genera and eight species were found in monthly captures during 24 months. From 471 wild animals tested for Leishmania infection, flagellates were observed in 56. Of these strains, only three were identified as Leishmania (all L.(V.) peruviana) -- one from Didelphis albiventris and two from Phyllotis andinum. Of 867 domestic animals (643 dogs, 198 donkeys, 21 mules and 5 horses) examined for lesions compatible with Leishmania, all were negative except one dog; and sequential parasitological examinations of the dog were all negative. Serological evidence of Leishmania infection was demonstrated by DOT-ELISA in eleven of 51 (21%) sera collected from dogs in the same valley (Guevara et al. Personal communication). Recently, Leishmania parasites have been isolated from three dogs in Purísima valley, and one dog from Canta valley (Lima department). We are now evaluating the potential role of dogs as reservoirs by transmission experiments. Despite our parasite isolations from D. albiventris and P. andinum, the role of sylvatic reservoirs remains largely unexplored.

The aim of Phase II is to develop an appropriate control strategy for Uta using the information generated through our studies.

The studies proposed below for Phase II, will develop and apply an appropriate control strategy for Andean Cutaneous Leishmaniasis in several endemic areas (in the departments of Lima and Ancash). In each of two valleys, disease incidence and a variety of entomological parameters are being recorded in four villages. In one village, all houses and peri-domestic walled structures (e.g. animal houses) will be fumigated with insecticide at strategic periods before the peak of vector activity (October and April). In a second village, as well as domiciliary fumigation, ravines and other sites that have been incriminated as resting places (probable foci of transmission) will also undergo focal spraying and will simultaneously be reforested. Reforestation with eucalyptus trees will be employed in order to change ecological features that may be necessary for the maintenance of Leishmania transmission. The third and fourth villages in each valley will act as controls -- one to experience reforestation (but not fumigation) and the other to be left completely unaltered during the course of the experiment. In all villages, we are

providing health education on leishmaniasis (plus other important health concerns), early diagnosis of lesions and free treatment. Forestation and fumigation activities will be conducted (under supervision) by the community, in conjunction with village health promoters who are being trained by our team.

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Evaluation of Projects Results: Discussion and Comments on Papers Presented

I. *Sherlock et al.*

Large fluctuations in the incidence of visceral leishmaniasis from one year to the next, clearly illustrates the need to have control areas when testing interventions. The presence of sylvatic *Lutzomyia longipalpis* has been confirmed in the study area. There may be a sylvatic leishmaniasis cycle with wild foxes as reservoirs, and human disease may occasionally spring from this cycle. *Lutzomyia longipalpis* prefers human blood to dog blood, but is mostly found after meals with dog's blood. This reflects the availability of the host. There is the impression that *Lutzomyia longipalpis* may be infected more rapidly from infected dogs than from infected humans. This has been indicated from xenodiagnosis performed on these two hosts. The efficiency of xenodiagnosis was discussed; it was felt that it is solely a research procedure which is not a useful diagnostic tool. The possibility of humans serving as reservoirs of visceral leishmaniasis in Jacobina apparently depends of high densities of *Lutzomyia longipalpis* being present. This occurs only for brief periods and human infection does not survive long enough for them to constitute effective reservoirs. If humans were good reservoirs, early detection and treatment would also be an appropriate means of control.

Leishmania chagasi has not been clearly differentiated from *Leishmania infantum*. The fact that opossums show limited pathology from infection with this leishmania may reflect recent introduction.

Double infection with *Lutzomyia mexicana*, *Lutzomyia chagasi* and *Lutzomyia brasiliensis* and any combination is possible. There appears to be no cross protection, because sequential infections are also occasionally seen. There is no data on protection or interference of species.

II. *Mutinga et al.*

Permethrin impregnated cloths work by killing insects not by repelling them or covering the inhabitants of homes where they are used. The insecticide is extremely safe and impregnation may last for six months. Non-repellent cloth colours, such as yellow or beige are preferred. The whole community has to use repellents to avoid infection of the neighbour who does not use the procedure. This method appears to be safer than spraying insecticides and the results may be longer lasting. An idea that may be applicable where hammocks are popular is to have them impregnated. A study of this has not been performed yet.

The role of sheep and goats as reservoirs of leishmaniasis is still being studied. Preliminary results found that 9 out of 900 goats in Westpokot were infected with *Lutzomyia ethiopica*. They may serve to transport leishmaniasis to new areas. The role of other animals was also discussed; dogs are probably the most important in this context. Lizards were found to be infected with a leishmania which so far has not been different from *L. major* and which infects small mammals.

Immigration which results from population increase and the search for available land is an important factor in the spread of leishmaniasis.

III. Hailu et al.

It is necessary to study epidemiological conditions closely since it is possible to have widely different patterns for visceral leishmaniasis in neighbouring environments, such as deserts and acacia forests. The impact of migration and resettlement on VL must be closely examined. Longer observation periods may be necessary to obtain clear results. There is enough information from some areas to indicate that migration into them has been influenced because of the endemicity of visceral leishmaniasis in other areas.

V. Weigle et al.

The specificity (99-100%) and sensitivity (95-96%) of the leishmanin skin test was evaluated prior to the study. However, repeated skin tests may sensitize a previously non-reactive individual. There was concern about the possibility of controlling forest exposures. Protection may include the use of more clothing and perhaps some types of repellents which are effective, acceptable and safe. Some risk factors would be better understood if the etiological fractions for them were calculated.

Studies on the population were performed every two weeks in order to detect early lesions. Migration between the areas being surveyed does occur and special forms were designed to account for this. Scars due to other causes were very frequent. Typical scars apparently indicated a greater susceptibility than the absence of scars. This was answered by several participants. Accounts of recurring lesions on traumatized individuals were recorded.

The economic implications of different interventions must be brought into the picture. Cost effectiveness is an important criterion for the selection of most procedures. Spraying a large area may be more expensive than treating a few cases in a subendemic area. Vaccination is still not an immediate solution.

VI. *Andrade et al.*; VII. *Calmet*; and VIII. *Rojas et al.*

The overall sense of discussion was that studies of the epidemiology of leishmaniasis and the specific identification of transmission sites, risk groups and risk factors is crucial for designing control strategies. It was apparent that transmission site and risk factors vary from country or region and that each region must follow standardized protocols for risk factor assessment in order to improve our understanding of leishmaniasis in different settings. The question was raised whether this was possible. A workshop on risk factor assessment is presently being planned under the WHO-TDR program.

The implications for control strategies resulting from these risk factors assessment studies were discussed. It appears that in Peru the risk factor migration is very politically and socially complex. The risk factor assessment in Mexico is promising but also not yet sufficiently complete to recommend specific control measures. The assessment of risk factors in Costa Rica is also preliminary and further analysis is required. Once analysis is complete, intervention in one community with one control community was proposed before making widespread recommendations. Even then, recommendations for control measures may need to differ from region to region, depending on the epidemiological, ecological and cultural setting. However, there is political pressure for widespread control measures.

Several questions regarding the details of the work presented by Dr. Andrade were raised. He provided the following information in response to these questions. On the question of cross reactivity of the Montengro skin test, he said it was highly specific, however, with respect to the ELISA test with *L. mex.* antigen, 6/7 sera from Chagas patients reacted. This cross reactivity may be avoided by raising the threshold of the test. Because Chagas disease is not endemic in the study area this cross reactivity is not a problem. The definition of chronic lesions was provided from endemic areas, clinically typical lesions in the ear(s), histopathology of an organized granuloma, in which parasites are absent. *Leishmania* are only rarely recovered from such lesions. However, these chronic lesions respond well to glucantime treatment. The longitudinal pattern of the humeral response is under study presently, so results are not yet available.

Dr. Rojas and Dr. Zeledon provided details regarding Costa Rica studies in response to questions; children under the age of 10 years comprised 45% of the reported cases in Costa Rica. Animal studies utilized the method of "Herrer", i.e. incubating tissue biopsies in antibiotic solution before inoculation. Dr. Rojas reiterated that his analysis was not complete, but that he had confidence in the trend of results thus far. He plans to analyze excluded and 29 included patients separately. Then, if they demonstrate similar associations he will combine the groups. Dr. Zeledon explained that *Lutzomyia* paralleled foci of *L. panamensis*, while *L. trapidoi*, although present in same foci, could not be implicated as a vector in Costa Rica.

XI. Perez et al. and X. Velez et al.

Although various questions were raised regarding technical details of the works presented, the active discussion of innovative vector control was uncovering methods mentioned by Dr. Velez including repellent impregnated curtains or bednets. He mentioned that nylon nets retained repellent better than cotton. To block sandfly entry, a very fine meshnet is needed. However, with impregnated nets large weave nets can be utilized permitting better airflow and are thereby more acceptable.

The use of Eucalyptus trees in the peridomestic areas was suggested as method needing evaluation. Dr. Llanos observed that sandfly captures were reduced in areas with Eucalyptus trees, even in settings of high sandfly densities. Dr. Perez explained in greater detail his research team's experience with the use of Eucalyptus oil extracts. He has observed that areas of the skin where such oil is applied have decreased sandfly biting. Unfortunately, adjacent skin areas lacking oil were still bitten. They have evaluated varying concentrations up to 20% and their preliminary findings were promising. This oil has been widely used for respiratory problems and dermatological conditions and therefore it is considered to be safe. Caution was mentioned regarding the use of Eucalyptus trees, because this tree consumes large amounts of water that could affect agricultural production.

Dr. Mutinga mentioned his experience with the use of a plant called "Neem" (Chinaberry). In Kenya an extract of the leaves and seeds of this plant was applied to a cloth and used against sandflies with promising results.

XI. Khoury et al. and XII. Llanos et al.

Discussion was primarily an interchange regarding questions about details of the projects. Dr. Saliba clarified that animals utilized in experimental infection were laboratory bred and of comparable sex and age distribution in the infected control group. Dr. Rachid commented that in Tunisia three methods are utilized for recovery of leishmania from zoonotic reservoirs, smear, serology and inoculation of skin samples into balb/C mice. Dr. Llanos clarified the methods they utilized to explore the relationship between rainfall, sandfly densities and the onset of new cases. Because the reported incubation period ranged from 2 months to a year, time series analysis was used iteratively to find the incubation period which produced the closest association, resulting in a one month incubation period. Therefore, one month was subtracted from the onset date to calculate the transmission data. He explained that the population was observed every 3 months for case detection. Incidence was 3-8%, prevalence of skin test reaction ranged from 40-80%. Extensive evaluation of wild and domestic animals was conducted over 24 consecutive months; 471 wild animal captures resulted in 56 flagellates, but only 3 were isolated from *Phyllotis adninnun* and 2 from *Dedelphis albiventris* possum. One-hundred and fifty (150) dogs were evaluated and 3 leishmania were recovered; PCR evaluation is in progress. In summary it appears that both wild and sylvatic reservoirs exist.

PANEL DISCUSSION #1:

"Leishmaniasis as a Public Health Problem and How it is Addressed Nationally"

Policies and Health Programs for the Study and Control of Leishmaniasis in Colombia

**By
Marcos Restrepo¹**

In Colombia, leishmaniasis has been considered a clinical entity since 1872, even though the parasite that causes it was isolated in 1929, taken from material coming from a mucous ulcer. The first reports recorded in Colombian medical literature refer to clinical descriptions of cutaneous and mucocutaneous lesions. The first case of visceral leishmaniasis was described in 1944.

American Tegumentary Leishmaniasis (ATL), is a zoonotic endemic disease widely spread in Colombia but generally considered of little importance in public health, due to several factors:

- a) health teams lacked knowledge about the disease;
- b) health units found it difficult to give an accurate diagnosis;
- c) patients' lives are almost never at risk, so it is tolerated by the community and overlooked by health institutions;
- d) there were no records showing the extent of the problem;
- e) as a result, there were no surveillance and control standards.

It was not until 1980 that the national government began to study and control tegumentary leishmaniasis, based on the experiences of a local program started in the province of Antioquia. The program confirmed the diagnosis of the disease at the beginning of lesions. Cases were duly recorded and now it is mandatory to report any leishmaniasis case which may appear. The national program provides specific treatment for most reported cases. Werner and Barreto reported 1865 cases of leishmaniasis from all over the country from 1979 to 1989, but only 283 of them had parasitologic verification.

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The information gathered has been collected by some publications, but there was no official or statistical data uniformly collected. A better understanding of the problem has been achieved by analyzing the information gathered as of 1981, when the first steps were taken to start a leishmaniasis study and control program. An analysis of Corredor et al. publication in 1986, helped determine that the annual incidence shows a rate of 3.90 cases in 100,000 inhabitants, which increases to 14.18 in 1990. The information available at the National Ministry of Health shows the amount of Tegumentary Leishmaniasis cases and the disease annual incidence in the country, as follows:

<u>Year</u>	<u>No. of Cases</u>	<u>Rate x 100,000 Inhabitants</u>
1981	1065	3.90
1982	1266	4.20
1983	1984	6.91
1984	2289	8.16
1985	1353	4.50
1986	1790	5.88
1987	2017	6.49
1988	2721	8.59
1989	4582	14.18
1990	3869	11.74

In this publication, the author's estimated rates are based on general population, since the specific population at risk was unknown at that moment. Leishmaniasis rates exceed those of other infectious diseases, such as diphtheria, tetanus, leprosy and brucellosis. Distribution by sex shows that men are more frequently affected than women in a 2 to 1 ratio. This incidence is mainly due to male labour situation and migration. Most cases occur in people over 15 years of age, who form the economically active group.

Distribution

Even though the disease is located in specific areas, the infection appears all over the country and coincides with most of the malaria affected areas, which cover 90.2% of the national territory. The disease has been reported in 29 of the 31 provinces in the country. It is centred around the following natural regions:

- a) the Pacific coast, formed mainly by rain forests, which represent one of regions with the highest rain fall in the world;
- b) the Atlantic coast, predominantly characterized by dry tropical forests;

- c) Catatumbo River basin, which extends beyond the border with Venezuela, mostly through rain forests;
- d) the Eastern part of the country, which is the third part of the national territory, made up of vast plains covered by dry tropical forests, rain forests, and jungle regions forming part of the Amazon territory that is shared with Venezuela, Brazil, Peru and Ecuador;
- e) the Magdalena River valley, which extends from South to North and divides the country in two zones, with dry tropical forests and rain forests.
- f) the Cauca River valley, which is also formed by dry tropical forests and rain forest.

All these natural regions are affected by leishmaniasis. The disease appears mainly in low regions that extend through mountains, up to 2,7000 meters above sea level.

The distribution of Leishmania species in Colombia was published by several authors, Saravia et al. in 1985 and Corredor et al. in 1990, and our studies in 1991, which show that the most frequently isolated species is Leishmania panamensis, widely distributed in the country. It corresponds to 53.8% of all the isolate strains, followed by Leishmania braziliensis, found in 30.3% of all the isolate strains. Concerning the Lutzomyia that causes the disease, Lutzomyia trapidoi is the most frequently captured species in the country.

Visceral Leishmaniasis

Visceral leishmaniasis in Colombia is restricted to several endemic regions and has less reported cases than tegumentary leishmaniasis. A few cases are reported every year and correspond to some endemic regions. Werner and Barreto's study indicate that up until 1979, only 20 cases of visceral leishmaniasis had been reported. Once the Visceral Leishmaniasis Program was started, more disease affected regions were detected and more cases were reported. The species Leishmania chagasi is considered responsible for the disease in Colombia. Disease affected regions are generally located in rural areas, except for a few peripheral areas of the cities that have rural characteristics. they are located in mountains that do not exceed 800 meters above sea level, have broken topography and form valleys between mountains, covered by dry tropical forests or rain forests. They usually have micro-climate that supports the Lutzomyia longipalpis, a vector, providing natural breeding places, mainly rocks. Occasionally the parasite colonizes artificially in good breeding places near human dwellings, barns, stock yards, animal pens, etc. This maintains animal to animal transmission, since the disease is characteristic in wild foxes and wild and domestic dogs; humans are a mere accident in the transmission chain.

Programs

Only in the last few years has leishmaniasis been given the importance that it really deserves. Cases have been duly recorded since case reporting became mandatory, this has shown the real incidence and extension of the disease. The existence of regions affected by visceral leishmaniasis, as well of their distribution, was also shown. It was found that ATL was highly distributed all over the country and that its predominant form is cutaneous more than mucocutaneous. It also showed that many patients suffer from severe malformation and even mutilation.

Nowadays, there is good motivation concerning the establishment of a leishmaniasis surveillance and control program, including the study of the affected regions. General policies were established to implement the programs in the country to guarantee integral attention for all the leishmaniasis affected patients. By 1987 the Antioquia Regional Health Service had gained a lot of experience in the handling and attention of patients, with well established laboratories and clearly defined technical and administrative standards that have helped the National Ministry of Health extend the programs to the rest of the country. This year, the Ministry of Health is discussing technical and administrative standards for the whole country.

Organization

In order to establish the program, the Antioquia Regional Health Service followed these steps and proceeded to:

- a) implement courses to qualify and permanently train the members of the health team;
- b) unify and standardize techniques, concerning patient diagnosis and handling;
- c) keep the required statistical records in order to determine the extent and characteristics of the problem;
- d) detect active and passive cases in order to know the real extent and incidence of the disease;
- e) analyze the origin of patients with positive diagnosis, in order to locate affected regions;
- f) have a permanent supply of required materials, especially laboratory supplies and specific drugs;
- g) develop and promote basic research for a better understanding of the disease, its handling, transmission and control.

The program established in the province of Antioquia measured the real incidence of the disease and served to compare this data to that from previous years. The incidence rate of tegumentary leishmaniasis in Antioquia increased from 3.5 per 100,000 inhabitants to 20.8 in 1990. In 55.8% of the cases it was possible to verify the diagnosis, and 88.9% of the patients detected by the program had cutaneous leishmaniasis.

Control

To control leishmaniasis is not easy, since it is a disease that stays mainly at wild levels, where several animal species are natural carriers, and man is an accident in the transmission chain. Several factors must be taken into account in order to control it. They include: the biology and ecology of carriers; parasite behaviour; vector characteristics; and the local ecology (fauna, flora, soil, meteorologic data, etc). Even the men that inhabit the region must be studied, concerning customs, migration and immunological characteristics.

The control strategies developed within the program established in Antioquia, and those designed by the national government, are centred around a few activities that aim to reduce contact between man and vectors. They are:

- a) To develop activities with community participation, in order to provide instruction in prevention measures using educational material;
- b) To identify the ecological characteristics of the regions where patients originate;
- c) To reduce contact between susceptible people and vectors, promoting net covered beds, screened doors and windows and the use of mosquito repellent and protective clothes;
- d) To promote the use of residual action pesticides inside and outside dwellings;
- e) To promote adequate disposal of garbage and organic material in order to reduce breeding places;
- f) To eliminate infected carriers when detected, such as dogs infected with visceral leishmaniasis;
- g) To conduct research in order to obtain new methods of control and efficient application.

Administrative Organization

The program is administered at different levels, which establish a network for the efficient and effective attention of patients. They are:

a) **Minimum level**: At this level the unit diagnoses most of the patients and generally does not have a laboratory. Its functions are to:

- detect affected patients and provide integral attention;
- take samples for direct tests, biopsies and cultures from the tissue and send them to the corresponding laboratories;
- prescribe adequate treatment in accordance with the parameters established by the program;
- refer patients to a more specialized level when diagnosis has not been established or when complications appear;
- support field studies in order to study affected areas;
- promote prevention and control in the community and educate people by means of talks, visits, brochures, etc.
- process statistical data and take care of the efficient handling of laboratory results;

b) **Intermediate level**: It is a health unit that has a laboratory. Its functions are to:

- fulfil the same tasks of the minimum level;
- do direct smear readings, according to the network or laboratory;
- send pre-read preparations for quality control;

c) **Regional level**: It administers a large geographic area and controls several minimum level health units as well as intermediate level units. It has laboratory and several laboratory technicians. Its functions are to:

- fulfil the same tasks of the intermediate level;
- take care of the adequate supply of drugs and materials;
- consolidate, critique and analyze statistical and epidemiologic information, gathered in the region and send it to the Superior level;

d) **Superior level**: It is made up of the offices that coordinate the activities provided by the Regional Health Service Division, specialized laboratories and university hospitals which are in turn coordinated by the Regional Health Service. Its functions are to:

- plan, program, supervise, evaluate and control the activities of the program in the corresponding province;
- serve as frame for all of the aspects related to patient diagnosis, attention and follow up;

- prepare reactivities and conduct specialized diagnosis tests;
- coordinate statistical and epidemiological analysis and critically review all information gathered by the program;
- guarantee adequate and timely supply of elements for the appropriate operation of the program;
- conduct studies of the affected areas, in respect to parasites, vectors and environment;
- send all information gathered by the program to the National Ministry of Health;
- conduct studies of the affected areas, with the help of minimum levels and regional hospitals.
- develop research projects.

The control measures should be based on long-term epidemiological studies of the foci, improve knowledge of the ecology and increase the effectiveness of research programs.

**Leishmaniasis as a Public Health Problem with Emphasis on
Latin America**

**By
Rodrigo Zeledón²**

Leishmaniasis is a group of important widespread diseases, apparently of ancient origin. They appear to be far more abundant and of greater public health importance than was previously recognized (WHO 1990). Leishmaniasis is not a notifiable disease in many countries and even in those where it is reportable, the actual number of cases is estimated to be three to five times higher than the number reported. The estimated worldwide prevalence of the different clinical forms exceeds 12 million cases, with an incidence of 400,000 new infections per year. The number of persons at risk has been calculated to be 350 million, affecting about 80 countries. Epidemics involving thousands of cases of cutaneous leishmaniasis (CL) have been observed in countries of Africa and serious epidemics of visceral leishmaniasis (VL) have occurred in Asian and African countries, resulting in tens of thousands of deaths (WHO 1991).

New settlements due to population expansion and new developments, such as industrial, agricultural or water resource projects, may bring non-immune persons into endemic areas which result in high numbers of new infections. Even the less aggressive cutaneous forms of the disease can produce serious socio-economic loss in terms of disability of the affected person and costly treatment which sometimes produces undesirable collateral consequences.

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In certain forest areas of South America, some development projects have faced serious obstacles due to fear of the workers of contracting mucocutaneous leishmaniasis and suffering its mutilating effects.

Recent discoveries particularly in Latin America, are demonstrating the great complexity of this group of diseases. Cases emerge in both known and new foci involving diverse ecological situations and including a great variety of parasite isolates, animal reservoirs and vectors. Clinical forms previously attributable to a single group of parasites, are now known to be produced by others which were previously associated with a different type of disease and vice-versa. Immunological impairment of different degrees, in which a given parasite plays a role, is becoming more evident, with different types of clinical pictures and resistance to treatment.

As an example of the above situation in the Americas, new foci are emerging in countries such as the United States (Texas) and the Dominican Republic. In the latter, 33 cases have been observed (H. Bogaert-Diaz, personal communication) of a very peculiar clinical form, produced by a new species of parasite. This new form of the disease was discovered only within the last 20 years and has some distinctive characteristics. The lesions are mainly nodules and/or plaques or hypochromic patches with no tendency to ulcerate; they are often disseminated, but some patients had only two or three lesions and seven had only one. The lesions are very rich in parasites and the Montenegro skin test is negative in most of the cases, indicating some sort of immunological impairment. At least two cases have cured spontaneously and others have responded to the intralesional injection of neoglumine antimoniate. This disease is probably also present in neighbouring Haiti where the suspected vector is present and conditions are similar to those of the Dominican Republic. In another Caribbean island, Trinidad, an enzootic cycle exists which at any time could become a true zoonosis involving man (Zeledon 1992).

Other situations that deserve special mention in the Latin American picture, are the findings of a type of cutaneous leishmaniasis produced by L. infantum in Costa Rica and Honduras and the identification of L. amazonensis as an agent for visceral leishmaniasis in Brazil.

In the first case, a nodular non-ulcerative form of CL sometimes presenting only papules or erythematous plaques has been reported, produced by a strain of L. infantum identical to zymodeme MON-1 of the Mediterranean area (Zeledon 1989; Ponce et al. 1991; Zeledon 1991). Some clinical forms are similar to the Dominican Republic cases, but parasites are scarce in the lesions and the anergic reaction to Montenegro test is not present in the Central American cases. Whereas in the Honduran situation, visceral cases occur concomitantly with the cutaneous cases, in Costa Rica only the latter form is observed. This difference may be explained by better sanitary conditions, with a lower child mortality rate and a lower malnutrition index in Costa Rica as compared to Honduras. A prospective study undertaken in Bahia, Brazil (Bardaro et al. 1986; Cerf

1987), suggested that both malnutrition and concomitant infectious diseases, acquired at an early age, are important risk factors in the acquisition of the classical picture of visceral leishmaniasis. Of 1,494 children with normal nutritional status, 12 had visceral leishmaniasis (8.03 cases/1,000). However, of 144 children with a moderate to severe degree of malnutrition, 10 had the disease (69.44 cases/1,000). This means that a child with moderate to severe malnutrition has 8.7 times more risk of developing visceral leishmaniasis. In the same area it was observed that a two year old child has a 1:10 chance of being infected and 1:4 chance of developing the disease, but if the child is malnourished the probability is 1:2. On the other hand, a seven year old child has a 1:6 chance of being infected but only a 1:36 chance of developing the disease.

In relation to the Brazilian situation it has been observed that L. amazonensis can produce a wide spectrum of clinical forms that include cutaneous (20/49 cases), mucocutaneous (5/13 cases) and visceral (11/45 cases), as well as four cases of post kala-azar dermal leishmaniasis and one diffuse cutaneous leishmaniasis (Barralet al 1991). The authors of these data have pointed out the need to re-evaluate the relationship of different species of Leishmania and the clinical picture they produce in humans.

These are just a few examples showing the complexity of the new trends of leishmaniasis in the New World. Finally, I would like to analyze, in a general way, the current situation of cutaneous leishmaniasis in a Central American country such as Costa Rica and how this country is addressing it.

The good sanitary conditions prevailing in Costa Rica have made possible an increase in the importance of leishmaniasis among parasitic diseases. The annual estimated incidence is 4,000-5,000 cases in a population of three million, making it the sixth most frequent of all notifiable infectious diseases. CL is widespread in rural areas; it is also associated with primary forest in less populated areas. The incidence tends to be higher during the rainy season. The most affected age group is under 10 years of age and it is common in children under two years. From 1982 to 1986 the mean annual incidence rate per 100,000 for the entire country varied from 55.2 to 116.8 and the highest rates were observed for the province of Limon, on the Atlantic coast, reaching 1,560.7 in 1985 and 1,355.0 in 1986. (Hidalgo et al. 1987).

Costa Rica has a good primary care health program with an important involvement of the community. CL is well integrated so that identification of cases is immediate and treatment mainly by intralesional injections of meglumine antimoniate is offered free of charge. This modality of treatment has proved to be very effective against L. panamensis, the agent responsible for practically all cases in the country, and its cost is lowered by a factor of 20. Awareness about the disease among the population also allows early detection of cases with mucosal involvement which, in any event, represents only 3-5% of the cases produced by the same parasite in Costa Rica.

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PANEL DISCUSSION #2:

"Appropriating Technology for Leishmaniasis"

On the Appropriation of Technology: Examples from Leishmaniasis and Health Research

**By
Humberto Guerra¹**

Technology in general is a response to problems, a search for improvements in the way of life, and is molded by the society and culture it originates from. Precisely for this reason technology transfer is a complicated issue. The most common case is one where a particular method, discovered and developed in one country (most likely an industrialized one) or region, is introduced or transplanted into another, a developing country. The term transfer already suggests that there is an agent, a "donor" which plays an active role on a passive recipient society. The problems of different peoples and societies may require different solutions, and only rarely will the same answer, without extensive revision, fit many situations. The answers to the local problems of developing countries must evolve and grow with the people who need those same answers, and they will have to modify any technology which may be available for "transfer". The developing society must choose what technology to develop: there is an investment, a cost attached with the effort of technology appropriation, even if it is only that of the loss of alternative technological opportunities.

The appropriation of technology is the process by which a group, an institution, a society or a country, after electing to solve a certain important problem, actively researches available technology and decides to acquire some particular ones, to make them their own, to develop answers to the problem. To this end, the group must learn, probably some of its members must receive training, and must develop expertise in all the aspects which are crucial to the application of the new methods. At the same time, the group must examine critically several different procedures, and evaluate the convenience of substituting those currently in use. In other words, the appropriation of technology is a general route to improvement, innovation, self-reliance, and development, and it is an active process for the developing country involved. The developing country is a "hunter-gatherer" of technology, until it can "cultivate" or "farm" it in its own soil.

The most obvious recent application of the appropriation of technology in health is the development of the primary health care strategy over the last few years. The

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general appreciation is that the famous goal, "health for everyone by the year 2000", will not be reached, but nevertheless most developing countries have been working seriously, building up their health structures, including infrastructure, personnel and methodologies, and important improvements are visible. More specifically, the attitude of the health establishments (Ministries and other institutions) themselves, toward the communities, the patients, and the tasks and duties of the health personnel has been changing, more and more toward prevention of disease and promotion of health at the community level.

Many influences have been shaping the training of health personnel and the definition of their work. Among them is the wide publicity that primary health care (PHC) has received through the media and publications in most countries, the use of didactic materials prepared by WHO and other agencies which emphasize PHC in most health personnel training activities, the demand by the communities themselves to be better served, the presence and work of non-governmental organizations (NGOs) at the community level, the existence of volunteer health promoters in many communities, and probably many more factors. The appropriation of technology in this case has been the grasping of the principles of the PHC strategy by many Ministries and health authorities and personnel in most countries, and the work toward the establishment of better health services, oriented to prevention and health promotion, in each of them. The strategy of PHC involves self-reliance, and therefore groups practicing it are highly committed towards finding solutions, through a very positive approach, and attempt to solve all possible problems. The activities performed as part of the human resources development have been many meetings, seminars, workshops, and training sessions and courses, most at local and provincial levels, but also some at national and international levels. A major characteristic has been the emphasis on learning by doing, and the reliance on in-service training. Exchange of activities and experiences in PHC has become a subject of technical cooperation between developing countries (TCDC).

In the case of leishmaniasis there are outstanding examples of appropriation of technology in Peru. The development of improved diagnosis, better epidemiological tools, an emphasis on extensive patient referrals and innovative changes in treatment and followup have meant a new outlook and prognosis for new patients.

The concentration of research efforts on leishmaniasis began in our Institute in 1977. At that time the emphasis was on isolation techniques, the development of culture media and appropriate use of antibiotics and antimycotics to avoid contamination of cultures from mucosal lesions. The adoption of hamster inoculation and standarization of hospital and field procedures followed. In the 1980s a routine was established for stock preservation and efforts for cloning and characterization by isoenzymatic procedures started. The use of DNA hybridization techniques was facilitated through a project emphasizing the development of nonradioactive methods, which culminated in a procedure based on biotinylated probes. Other projects exploring transmission of leishmaniasis in the communities in endemic areas were enriched by improved isolation and characterization procedures and greater involvement with entomological work.

Serendipitously, certain cloned segments of minicircle kDNA, when sequenced, yielded primers for a Polymerase Chain Reaction (PCR) able to recognize Leishmania belonging to the braziliensis complex.

All the while exploration of endemic areas and collection of more strains continued, epidemiological observations in selected areas began to involve behavioral risk factors and social aspects. The commitment to provide services to the communities being studied derived in adoption of simplifications of ambulatory treatment, specifically use of intravenous pentavalent antimonials, and lately intralesional treatment of cutaneous leishmaniasis, with improved compliance, reduction of the treatment period, and better final results. Serological efforts using the ELISA format began to give interesting results, with improved sensitivity and specificity than experienced with other procedures.

Clinical classification of leishmaniasis cases on the basis of the speed of involvement of skin and mucosas showed that antimonial treatment was less effective in the most affected patients, those where the disease had progressed more rapidly. The only treatment for these refractory patients is Amphotericin B, which is too toxic for ambulatory use. Experimental treatment with Amphotericin B-Lipid Complex resulted in much reduced toxicity, but high relapse rates. The PCR technique has been tested under field conditions in endemic areas, and found to provide dependable results in a very simplified version. The incriminated vector of Andean cutaneous leishmaniasis, Lutzomyia peruensis was shown to harbor Leishmania peruviana, which was biochemically characterized. Treatment of patients in endemic areas has been established by the Health authorities, with participation of leishmaniasis patient organizations, non-governmental organizations, and large groups have been treated under close supervision to observe the results of therapy. Adoption of immunotherapy is under consideration.

This overview of the development of research efforts in leishmaniasis has outlined some of the more significant results. The most important result is that workers in the laboratory, the clinic, and the field are all keenly aware of results of ongoing efforts, and are constantly contributing to them. The new technologies which arise elsewhere are subjected to scrutiny, their possible value if adopted is considered, and may be tested at a small scale level. Constant discussion and participation in meetings and workshops with the Health authorities and the control organism of the Ministry of Health has generated an atmosphere of cooperation in many aspects.

The appropriation of technology through an active group of researchers, as exemplified by what is being done in leishmaniasis, is a continuous, ongoing process which is capable of yielding results in attention of patients, development of scientific knowledge, institutional strengthening, individual advancement of scientists, increase of training capacity, and should allow the establishment of prevention and control procedures.

Immunoenzymatic Method Dot-ELISA in the Detection of Potential Reservoir Hosts of Leishmaniasis in Peruvian Rural Endemic Regions

By

Luis A. Guevara² and Adriana Paredes²

Introduction

To succeed in the control of leishmaniasis, the study and analysis of the vector, parasite and reservoir host must be done. Since all the New World Leishmaniasis are zoonosis, to better understand and assess the control of this disease, serological studies of domestic and wild potential reservoir hosts, in endemic regions are essential.

Diagnostic methods have been applied in leishmaniasis research but some methods which are appropriate for reference laboratories tend not to be appropriate for use in rural areas, where the disease is endemic. While serodiagnostic methods similar to those used in human leishmaniasis can be applied to the study of wild and domestic potential reservoirs, difficulties of conducting serodiagnosis in the field impairs such studies. There is a need to find an adequate and reliable method to work with.

Work has not been done at the serological level in reservoir hosts in Peruvian endemic regions. To know the existing condition in potential reservoir hosts, and applying an improved low costimmunoenzymatic method, Dot-ELISA, we carried out a survey among dogs living in endemic regions with this method.

Since 1913 when Pedroso documented the first infected dog in Peru many other experimental studies, at the parasitological level, have been undertaken in endemic regions. Opossums, rats, foxes, edentates, armadillos, horses and dogs have been studied to isolate of parasites. Attempts to detect infected animals, as evidence of active peridomestic transmission, have also been conducted. Despite the complexity of the risk of infection and the evidence of the parasite, the serological study of reservoir hosts in endemic regions can complement parasitological studies, giving an initial indication of the situation in situ.

Serodiagnosis

Serology is widely used in the diagnosis and research of human leishmaniasis, and to detect antibodies, in vitro, against the parasite. There are several available methods, varying in complexity and cost: Immunofluorescence Antibodies Test, IFAT; Complement Fixation Test, CFT; Immunoenzymatic Assays, ELISA; Direct Agglutination Test, DAT. The specificity and sensitivity of the methods depends on several factors such as antibody

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concentration in sera-severity of the disease, nutritional status of the host and the utilization of specific, partially purified or characterized leishmanial antigens.

It is known that improved serological methods would help in studying the risk factors and reservoir epidemiology. Applying some of these methods to perform a survey detecting antibodies against leishmania parasites in dog's sera would be useful in the search of potential reservoir hosts in places where these animals may play an important role in the dissemination of the disease.

There are simple and sophisticated methods. However there are difficulties to be solved first before the survey starts: the stability of reagents, the high cost of the laboratory equipment and the lack of well trained workers. At our laboratory, we had improved a low-cost, reliable, versatile immunoenzymatic method to detect antibodies against leishmania to be used in the field.

Diagnosis at Field Level

Working in serodiagnosis in the rural areas, one faces unpredictable technical and physical difficulties. Therefore, to achieve ones goal, one should be able to solve all of them at once.

In many Peruvian rural endemic areas, facilities to undertake such work are difficult: very bad roads, no power supply or distilled water. Variable climatic conditions are an additional stress with very hot, high humidity in the jungle and cold and dry conditions in the Andean region. Working with delicate reagents, antigens, conjugates and immunologicals under these difficult conditions, requires extreme care.

Faced with these obstacles, and trying, in some way, to understand the risk of infection and the dissemination of leishmaniasis, we improved an immunoenzymatic method, originally reported by Pappas in 1984 to detect antibodies against *Leishmania* parasites in sera.

The antigen: A culture of *Leishmania braziliensis braziliensis* (MHOM/BR/5/M2903) growing in bifasic medium was prepared according to Romero *et al* (1987). The parasites were washed three times in sterile phosphate buffer saline (pH 7.4) and brought to a concentration of 5×10^6 /ml. The parasites were dotted onto nitrocellulose filter paper disks of 5 mm in diameter and put into microtiter plates of 96 flat bottom wells. The microtiter plates, containing the disks with the fixed parasites, were carefully kept, sealed in plastic bag under vacuum with silica gel, and stored at -20°C until use. The antigen, kept in this condition, under normal laboratory conditions, was recognized by its corresponding antibody without losing reactivity for up to one year.

Sera and conjugate: The sera collected from the individuals in the field were mixed with sodium azide 0.01 % and then stored in the dark until use. When possible the samples were kept at -20°C with a small difference in the final chromogenic reaction. For each assay, positive and negative control sera were used. The conjugate used in this assay was anti-human IgG alkaline phosphatase purchased from SIGMA. The conjugate was dispensed in small, "ready to use" plastic vials.

Dot-ELISA Procedure: The disks dotted with the antigen, were treated with HCl 0.1 N for 30 minutes and washed twice with phosphate buffered saline (PBS pH 7.2). The disks were blocked with PBS-Tween-20 0.05 % containing 8% non-fat milk and incubated for 45 minutes at environment temperature (ET). Disks were washed three times with PBS for 10 minutes. Serum samples at several dilutions were prepared using PBS-Tween-20 containing 2% non-fat milk and incubated for 45 minutes at ET. The microtiter plate containing disks were washed with PBS, and the conjugated anti-human Ig G at 1/800 dilution was added.

The microplate was incubated for 45 minutes at ET, washed three times and incubated for one minute only with NaCl 1.16 M. The plates had a final wash three times with PBS-Tween-20. The substrate was prepared using 4.8 mgs of nitro blue Tetrazolium and 2.4 mgs of bromo-chloro-indolil phosphate, dissolved in 100 µl of N-N dimethyl formamide, and brought to 10 ml of alkaline phosphate buffer (pH 9.5).

By washing the microplates with distilled water the enzymatic reaction was stopped. The positive samples showed a dark blue spot on the white paper, and the negative samples showed no visible colour.

Results and Discussion

Ninety sera samples from dogs were collected by the field team lead by Dr. Alejandro Llanos from Huayllacullán Valley in the midst of the country where leishmaniasis is endemic. Twenty-five percent of the collected sera were positive by the Dot-ELISA. Our positive working dilution, after filtration with the positive and negative control, was 1/100. This dilution was used as a limit to distinguish the positive from the negative sera. However, this dilution could be replaced by the next immediate dilution (1/200) to sharply distinguish the samples with high antibodies concentration and diminish the chance of cross reaction. At this dilution the variation between the positive sera was not significant because 23.3% of the samples with this dilution were also positive.

We should mention that the feces of dogs in this study were not examined for parasites or other infectious agents. Because antibodies against worms and other parasites can interfere, giving cross reaction with leishmania (Luis Guevara unpublished results) this may affect the real status of infection.

To avoid the increase of false positives, we are in process of searching for specific antigens to diminish or eliminate the risk of having high percentage of positive samples. Preliminary results obtained in collaboration with Dr. Angel Hernandez in Caracas show the strong possibility of having a glycoprotein with molecular weight around 50-52 Kd to use in the diagnosis and recognition of an infected individual.

Since the risk of human infection cases by leishmania parasites could increase, as long seropositive cases of infected dogs in endemic regions exists, the control of the reservoir hosts should be pursued. To reduce the risk of transmission of leishmaniasis by wild animals, the majority of the potential reservoir hosts should be tested. In the Amazonian region this should include primates, opossums, armadillos, rats, etc.

Having a versatile and low cost (each sample costs \$0.20 USD) immunoenzymatic method, the study of prevention and control of leishmaniasis looks promising. This serodiagnostic tool for use in the field work is ready at our laboratory in a kit design and should be available for the peripheral field worker soon. It must be emphasized that these diagnostic testing kits should be taken where the disease is, and it can be applied to other diseases.

This very preliminary and first report in Peruvian endemic regions shows a relatively high percentage of positive dogs living in endemic areas. These results demonstrate the importance of developing diagnostic tests for the study, prevention and control of the leishmaniasis in Peruvian endemic regions.

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PANEL DISCUSSION #3:

"Adapting Leishmaniasis Treatment to Peripheral Health Centers and Communities"

Adapting Anti-Leishmanial Therapy to Peripheral Health Services and Community Health Workers

By

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Introduction

The clinical goals of any therapy should include curing the patient, preventing relapse, avoiding metastatic disease, reducing unresponsiveness to currently available medications, and keeping treatment costs, including hospitalization if applicable, to a minimum. In general, and especially in the case of the leishmaniasis, a serious concern of those providing health care, as well as of those performing research, must be also to make treatment acceptable, affordable, and accessible.

Limitations in the Administration of Anti-Leishmanial Therapy

In Peru, as in most of the countries where the leishmaniasis are endemic, it is the population of rural communities, where the health care coverage is usually only partial and frequently absent, which is most affected by the disease. A number of factors make access to appropriate treatment in these areas difficult to say the least.

The first line drugs of choice for all forms of leishmaniasis, except diffuse cutaneous leishmaniasis, remains the pentavalent antimonials (WHO 1991). Currently, the main problems with the use of these drugs include:

- the high cost of the commercial preparations of the drugs;
- the need to administer the drug through intravenous or intramuscular injection, in prolonged therapeutic schemes;
- the relatively frequent occurrence of side effects, especially in communities which are most affected by the disease;

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- the need for trained personnel to provide both clinical evaluation and treatment to patients.

A study of the Purisima valley, an endemic area of Andean cutaneous leishmaniasis (Uta), serves to illustrate the common situation. In this valley, the community knowledge, attitudes and practices related to leishmaniasis therapy were assessed through a survey. Some of the results are summarized in Table 1 below.

Table 1. Knowledge, attitudes and practice on leishmaniasis therapy of the population in an endemic area of Uta*.

Treatment	Knowledge of n (%)	Best choice n (%)	Has used n (%)
Antimonials	48 (71.6)	37 (55.2)	25 (37.3)
Herbs	45 (67.2)	7 (10.4)	37 (55.2)
Physical substances	3 (4.5)	0	3 (4.5)
Chemical substances	33 (49.3)	7 (10.4)	22 (32.8)
Ointments	4 (6.0)	1 (1.5)	1 (1.5)

* Responses by 67 randomly selected family heads who were interviewed.

Despite most respondents belief that antimonial drugs were the best choice for treating cutaneous leishmaniasis, they frequently used chemical substances and herbs. Furthermore, when antimonials were used, very low dosages were administered. Before our group began working in this area, no patient within it had received an adequate course of treatment (as currently recommended).

Two of the most probable reason for this apparent gap between knowledge and practices is the scarcity and high cost of pentavalent antimonials (approximately US\$ 150 for a 10 days course of treatment in an adult of 60 Kg of body weight; unaffordable for many of those suffering from the disease). This causes the population to inadequately self-medicate and/or to use inefficacious traditional or other treatments.

It is important to note that most of those interviewe (46/67) acknowledged that they had administered some kind of anti-leishmanial therapy either to relatives or to friends. Although the prescribed therapies included the use of pentavalent antimonials generally it was inadequately administered.

In general, we have found this practice to be widespread among populations of endemic areas. The history of anti-leishmanial therapy prior to admission in out-patient clinics for patients with leishmaniasis between 1984 and 1990 is summarized in Table 2 supports this observation. Nearly 25% of the patients with cutaneous disease and 50% of those with mucosal involvement were treated with antimonials before admission date. However, the majority of these patients had received irregular and insufficient treatment.

Table 2. History of previous anti-leishmanial therapy with pentavalent antimonials administered in endemic areas

Clinical form	Number of patients	Age Years	Prior Therapy		
			Drug	No.	Inadequate*
Cutaneous	188	14-70	Sb ⁵	23	16 (69.5%)
			Sb ³	22	15 (68.2%)
Cutaneous	47	<1-14	Sb ⁵	5	5 (100%)
			Sb ³	7	7 (100%)
Mucosal	81	15-61	Sb ⁵	41	23 (56.1%)

* Inadequate because irregular (no consecutive days) or insufficient (less than 10 mg/kg/day) drug administration.

In all cases, the improper use of the pentavalent antimonials produces a low success rate, as a result of incorrect administration routes, and inadequate number of courses and dosages. The implication of this is significant for any control programme.

On the other hand, health workers, when present, usually lack knowledge about the disease and its appropriate management. Table 3 shows the result of a pre-test applied to health workers attending a training seminar offered by our group.

Additionally, there is a lack of stimuli and of logistic support, compounded by a rather discriminatory promotion system among health workers.

Table 3. Results of the pre-test applied to health workers* on general knowledge on leishmaniasis

Subject	Total	Knowledge	
		Adequate	Not adequate
Disease	29	18 (62.1%)	11
Parasite	29	5 (17.2%)	24
Vector	29	4 (13.8%)	25
Reservoir	29	11 (37.9%)	18
Transmission	29	10 (34.5%)	19
Control	29	1 (3.5%)	28
Treatment ¹	29	17 (58.6%)	12
Treatment ²	29	2 (6.5%)	27
Relapsing	29	6 (20.7%)	23
Drug/vi	29	22 (75.9%)	7

* 1 physician, 1 technical supervisor, 7 lab technicians, 16 health technicians and 4 health promoters

¹ : first line drug (no dose)

² : second line drug

It has been found that the success rate of therapy can depend very much on the way it is provided. Table 4 shows the difference between the cure rates for cutaneous leishmaniasis achieved by a supervised and a non supervised scheme, both administered by trainee nurses using the same therapeutic dosage (Castro and Jimenez 1988).

Table 4. Difference in cure rate obtained by supervised and non-supervised therapy*

Result of therapy	Supervised therapy	Non-supervised therapy
Clinical cure	13 (86.7%)	2 (13.3%)
No cure	2 (13.3%)	13 (86.7%)
Total	15 (100%)	15 (100%)

Ref.: Castro S. & Jimenez E. 1988

* both prescribing 20 mg/kg/day of Sb* for 20 days

The study suggests: an important loss of drug, due to misuse or diversion; an increase in the probability of the emergence of resistant strains; an increased probability of mucosal involvement where MCL exists due to therapy abandonment; and the loss of credibility by the health services may occur altogether.

The need to overcome these difficulties can not be overemphasized, and appropriate strategies must take into account both the community and the health services. What is needed for the community is health promotion and education to encourage the early pursuit of appropriate treatment. For health workers, training in currently recommended therapy, as well as in appropriate design and management of supervised schemes may be considered as necessary.

Action Taken by the Leishmaniasis Working Group (LWG)

The LWG has taken different actions to improve the situation in the study area. First it adopted a supportive attitude towards all levels of health workers involved in the management of leishmaniasis, with emphasis on those working in the field within endemic areas. In more practical terms this involved training health workers including physicians, nurses, health technicians, laboratory technicians, and health promoters, either working within the Ministry of Health (MOH), non-governmental services, and the organized community. It should be recognized that antimonials are used at the community level and that it is therefore appropriate to consider training community representatives in their use.

In coordination with national and regional health authorities, the LWG participated in or directly implemented training courses, seminars, and workshops. Consultancies and advisory activities are continuously performed in favour of the health authorities and workers. Members of the LWG include co-founders of the National Committee for Leishmaniasis Control, a consultant/advisor board to the Dirección Nacional de Control de Malaria y Otras Enfermedades Metaxénicas (DNCMOEM - National Direction for Control of Malaria and Other Metaxenic Diseases), and participate in similar other boards at some regional levels.

The medium and long term results of these actions have been frequently disappointing in the case of interaction with MOH health workers. Some of those who did begin to implement or to participate in control activities (basically diagnosis and treatment of patients) were shifted to other positions. In other cases they were dedicated to activities not related to leishmaniasis control, particularly in the case of laboratory technicians.

However, there have been positive results. In Ambo, Huanuco, an area with one of the highest incidences of leishmaniasis in Peru due to *L.(V.) braziliensis*, a local control programme based in the MOH district health centre has been active for the last

three years. This has been achieved the active participation of a member of our group, who remained in the area for four months training and helping local personnel to implement control activities.

In the case of the DNCMOEM, collaborative action has achieved significant goals. Many results or findings relevant to leishmaniasis control have reached practical application, and coordinated action with several other institutions and the help of international aid has obtained the largest supply of drug ever available in Peru for a single year.

Given the above and the changes currently occurring in the Peruvian state organization, including the MOH, which has resulted in a transference of responsibility and decision making to regional and local levels, we are now implementing a different strategy in our collaborative relationship with the MOH services.

In the future, training and advisory activities will be based on agreements signed with regional/local authorities in charge of health care in endemic areas, who will also assume responsibilities, such as ensuring support for their health workers and giving the due importance to leishmaniasis as a health problem.

To date, with emphasis on this policy, personnel from two "territorial health units" (covering the eastern and northern part of Lima) have participated in a basic training course, and have been provided with manuals and guidelines. Their principal tasks have been identified and their progress will be assessed in quarterly evaluation/supervision meetings. Coordination for implementing the same action with personnel from Sicuani (Cusco) and Sandia, Azangaro, and Juliaca (Puno) are currently underway. At the same time, a proposal for similar activities with a wider scope is being worked out with the national level health authorities for leishmaniasis control.

In the case of community health workers, although we have worked with a rather small number of health promoters, the picture is more satisfactory. For example, in the case of the Purisima Valley, two young female community health workers have been progressively trained "*in service*", while they worked along side LWG members. Currently, they are proficient in tasks such as case detection, sampling procedures for diagnosis and parasite isolation, and treatment administration. Today, they are in charge of routine control activities in the valley. The LWG supervises their work every three months and provides them with materials, drugs, and minimum financial support for local transportation and other expenses. This type of activity is basically performed where the LWG conducts fieldwork.

Controversial Subjects

Over the course of our work in Peru a number of subjects have proven to be controversial. For discussion purposes some of them are listed below:

- Drug:** availability, scarcity
cost... who should pay the price?
To what extent can drugs be put in hands of the community?
safety, etc. ...
- Priorities:** disease form and, individual patients, groups of patients, communities, endemic areas, etc.
- Diagnostic tools :** real need for these to be included in case definitions and for therapy decision making, case management, etc.
- Social aspects:** their relevance and the possibilities to intervene upon them
- Role of:** Government (MOH) at national, regional, local levels
Research and academic institutions
Community
Non-governmental institutions

In general there is no single answer. Any solutions will depend on the particular characteristics of the problem in each place.

Conclusions

We have identified a number of factors important for the success of collaborative action with those responsible for health care (either from the MOH, the non-governmental services, or the organized community). In summary it is important to:

- ensure that priorities for researchers and those responsible for health care- are justified and compatible;
- provide knowledge on the disease;
- provide knowledge and training on correct therapeutic schemes, and control measures;
- pursue the above with a training "in service" methodology;
- provide support for periodic supervision and follow up, performed in coordination with those in charge of local/regional authorities.

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Adapting Leishmaniasis Treatment to Peripheral Health Centers and Communities

**By
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Introduction

Rural health posts represent the first level of formal medical care, and the majority of patients make their first contact with the health authorities at this level. Their geographical location and the personnel that staff such posts helps give them an informal character which represents an important starting point in the interaction between the community and the formal health care system.

The implementation of health care at this level has optimized the efficiency of measures such as oral rehydration and the treatment of infectious disease. The participation of community health workers in diagnosis and treatment is increasingly important, particularly as related to acute respiratory infections (1) and tuberculosis (2). The involvement of mothers' groups in self-care of family and community health is another notable example of community participation (3).

Treatment of Leishmaniasis

At present the drug of choice for treatment of all leishmaniasis, with the exception of the diffuse cutaneous form (DCL), are the pentavalent antimonials (4). Two formulations are available, (i) meglumine antimoniate and (ii) sodium stibogluconate, of which the former, marketed commercially as "Glucantime", is most used in the Americas. The drug is supplied in 5 ml ampoules, each containing 85 mg/ml of pentavalent antimony (Sb^{5+}), and is administered intramuscularly (IM) or intravenously

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(IV). The dose recommended by the World Health Organization is 20 mg of Sb³⁺ per kilogram body weight per day, divided into two injections. The maximum recommended adult dose is 850 mg, i.e., 2 ampoules (5). Leishmaniasis treatment is administered until clinical cure of lesions is achieved in cutaneous forms or parasitological eradication demonstrated in mucocutaneous or visceral forms of the disease. The average duration of treatment is 4 weeks, although this varies.

Availability of Treatment

In Colombia, glucantime is obtained through the government from national or foreign laboratories. The quantity provided to each hospital is dependent on the number of cases registered with the Ministry of Health, the official body concerned with distribution of the drug.

Patients diagnosed as having leishmaniasis generally are admitted to the local hospital for treatment, either by the local primary health post or community volunteer. The patients access to these local hospitals for diagnosis and treatment is limited by several factors, the most important of which are distance, travel costs (transport, food and lodging) and reluctance or inability to take time off from work. Ignorance of the potential consequences of leishmaniasis and belief in folk remedies are other limiting factors (Fig. 1). The consequences of this are as follows:

1. Late consultation and diagnosis may result in chronic, extensive and disfiguring lesions (Cutaneous leishmaniasis-CL); mucosal involvement (Mucocutaneous leishmaniasis-MCL); and general malaise and death (Viseral leishmaniasis VL).
2. Irregular treatment due to non-attendance of the patient at the health care center may prolong the curative process, increasing discomfort, toxic side-effects and greater cost as greater quantities of the drug are needed to counter irregularity of application. As these factors increase, "compliance" of the patient may fall correspondingly, resulting in the total abandonment of treatment.
3. Increased diagnostic costs. The parasitological tests used in the etiological diagnosis of chronic cases are more sophisticated and costly than standard methods (6).

Adaptation of Treatment to the Local Health Post Level

The advantages offered by diagnosis and treatment of leishmaniasis at local health posts are shown in chart 1. The ready access to such facilities allows patients to be treated on a frequent and regular basis. This lowers the costs of diagnosis and treatment as well as the expense to the patients themselves. The duration of treatment is reduced and patient compliance increased. Early treatment limits the severity of the disease and reduces the risk of complications. Community awareness and participation are also increased.



FIG. 2

CHART 1

**ADAPTATION OF TREATMENT AT THE
LEVEL OF LOCAL HEALTH POSTS:
ADVANTAGES**

Variables	Treatment	
	Hospital	Health Post
Early detection of cases	low	↑
Costs (government and patients)	high	↓
Compliance	low	↑
Duration of treatment	prolonged	↓
Morbidity (severity)	high	↓
Community participation	low	↑

Obstacles

We believe the main existing obstacles to the the adaptation of leishmaniasis treatment in local health posts are related to three aspects: diagnosis, treatment and administrative factors.

1. Diagnosis

The symptoms produced by visceral leishmaniasis are not sufficiently specific to differentiate it from malaria and other generalized infections, while lesions due to cutaneous leishmaniasis present a wide spectrum of clinical forms and may resemble other pathologies. In mucocutaneous leishmaniasis patients, the presence of a scar as a result of a primary cutaneous lesion may serve as an indicator for diagnosis.

The criteria for clinical diagnosis of leishmaniasis vary considerably according to the region, the parasite and the expertise of the health workers carrying out the diagnosis, so it is difficult to define general diagnostic guidelines. The identification and isolation of the parasite (parasitological diagnosis) is the only way to confirm a diagnosis of leishmaniasis. The most sensitive methods require a health care infrastructure and trained personnel and as such are expensive, beyond the normal resources of any local health post. While certain parasitological techniques can be applied to local health care posts these tend to be much less sensitive and specific (7).

2. Treatment

There are variations at the local level in the amounts of glucantime administered to patients. Smaller doses than those recommended by the WHO are often applied, giving subtherapeutic levels that prolong treatment and worsen the patient's condition. The reasons for this include ignorance of the correct dose required, insufficient availability of glucantime and discomfort of the patient caused by the injections. On the other hand, even glucantime therapy administered according to WHO guidelines presents certain problems: multiple doses over a prolonged period are required; the drug must be applied parenterally by qualified personnel; the risk of viral infections acquired from re-used syringe needles; and the sometimes incapacitating effects of the treatment. Each of these factors lower "compliance" of the patient. Glucantime therapy is also costly. Treatment of an adult with cutaneous leishmaniasis requires an average of 50 ampoules at a total cost of \$150 US, not including other medical expenses.

3. Administrative aspects

Communities affected by leishmaniasis have had little or no participation in the diagnosis or treatment of the disease up to the present time. These activities have traditionally been performed exclusively in local hospitals which have rarely sought more active participation from the communities they serve. In many places the diagnostic and

treatment program is governed by the availability of glucantime and is performed in an improvised or disorganized manner. The absence of technical guidelines has impeded the standardization of diagnostic methods and treatment up to the present time.

A great many of the medical and paramedical personnel assigned to Leishmania-endemic areas have little prior experience of leishmaniasis and lack fundamental knowledge of its diagnosis and treatment. At present only those patients who arrive at the hospital receive glucantime therapy (passive detection), and many more who have no access to health care go without treatment.

In spite of the fact that leishmaniasis in Colombia is a disease that by law has to be registered, many cases still go unreported and the true incidence of the disease is probably much higher than estimated. Under registration of cases by medical personnel unaware of its importance is one reason why hospitals often lack adequate supplies of glucantime, cost being another factor. Diagnostic and treatment programs for leishmaniasis have lacked a separate budget in the past, forcing them to compete with other health problems accorded greater priority.

Search for Solutions

1. Administrative aspects

Efforts should be geared towards the creation of a level of primary health care for leishmaniasis patients. This should be readily accessible to patients and ensure that they receive prompt and adequate treatment. An infrastructure exists in most countries for primary health care, with trained medical and paramedical staff available to treat leishmaniasis patients. This reduces the costs of a leishmaniasis control program.

A specific budget should be set aside to cover administrative overheads as well as the costs of parasitological examinations, active search for cases and education programs related to leishmaniasis. An interesting alternative would be to allow health posts to receive glucantime directly from the manufacturers at a reduced price, and to allow them to distribute the drug within their areas as necessary. Government support for these measures is important but the willingness of local communities to participate, and their capacity to organize, propose solutions to their health problems and take responsibility for the consequences are probably more important.

2. Diagnosis

Sensitive, inexpensive and simple diagnostic methods should be developed and adapted to local conditions for use by trained paramedical personnel. The establishment and standardization of clinical criteria for the diagnosis of different forms of leishmaniasis would facilitate the work of paramedical staff and health volunteers in the detection and treatment of the disease, as would the development of protocols and clinical tools that can be used in health posts.

Implementation of certain parasitological diagnostic methods is already feasible from the technical point of view. Nevertheless, the ability of health workers in particular communities to implement and perpetuate these techniques should be ascertained.

The early detection of suspected cases of leishmaniasis and their referral to health posts is a task that can be fulfilled by the community. Development of clinical and parasitological screening methods for use by paramedical personnel is another priority requirement.

One instrument (the prediction rule) that was developed by CIDEIM researchers to permit the diagnosis of American tegumentary leishmaniasis (ATL) in endemic areas where parasitological diagnostic methods are not possible. An evaluation and categorization of a series of clinical and epidemiological variables in the patient, allowing a conclusion, is expressed as a final score. If this score exceeds a certain threshold value the probability that the patient has ATL is very high.

The rule was developed from studies of a group of patients, most of them from the Pacific Coast of Colombia, of which 100 had ATL confirmed by parasitological methods and 38 displayed other etiologies. The variables that were finally selected to develop the rule were:

1. Physical examination

- * type of lesion
- * lymphadenitis
- * trophic changes

2. Clinical history

- * history of trauma
- * origin in endemic area

3. Intradermal Montenegro test

Results in the group used to develop the rule were: sensitivity 96%, specificity 92% and efficiency 95%.

To validate the usefulness of the rule a second population was evaluated, of which 123 patients had ATL and 35 some other etiology. Results here were: sensitivity 96%, specificity 70% and efficiency 87%.

These first results indicate that the prediction rule allows the detection of ATL patients without recourse to parasitological tests. The rule is an instrument that can be used by paramedical personnel and health volunteers.

3. Treatment

Most problems related to the treatment of leishmaniasis are concerned with glucantime. Efforts should be made to seek a better method of treatment. In the long term this method should satisfy the following criteria:

- * Efficacy equal to or better than the pentavalent antimonials
- * Administered orally
- * Only one dose required
- * Free of unpleasant side effects
- * Inexpensive
- * Does not require medical supervision

In the short term, several promising alternatives are available, notably the intralesional application of glucantime. The advantages of this treatment are: less drug and fewer contacts with patient are required, reduced adverse and toxic side effects, increased compliance of patients, and reduced cost of treatment

The disadvantages are as follows:

- * Still requires trained personnel
- * As yet no clinical trials have demonstrated a greater effect than standard IM or IV application
- * No studies have demonstrated efficacy in the treatment of cases where there is a risk of systemic involvement

Conclusions

Leishmaniasis is a disease that mainly affects rural inhabitants who have limited access to treatment by trained personnel. Thus, the adaptation of methods of diagnosis and treatment to health posts is crucial for early and adequate management of the disease. While government involvement is important, active participation of affected communities is essential.

At the local level, the critical points in achieving early, accurate diagnosis, complete treatment, and the effective administration of resources for leishmaniasis control should be identified. Strategies and methods can then be tailored to meet the priorities of local communities, which are necessarily increasingly involved in the decision-making process.

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DISCUSSION SUMMARY

Dr. J. Chang pointed out that in Peru, the high cost and scarcity of antimony resulted in the use of alternative therapies of unproven benefit. Even in the case of antimony treatment, there was a high incidence of inadequate dosing. The reasons for non-compliance are certainly complex, but not the least of these would include patients selling at least a portion of their allotment of drug for economic gain. This leads directly to the recommendation for better training of local health workers and more rigorously supervised chemotherapy. The larger question of course is when and under what conditions should drugs be put in the hands of the community? Overall, it seemed clear that if adapting leishmaniasis treatment to peripheral health centers and communities was to be successful, there is a need for more sophisticated local, rural health units where appropriate supervision, education and training can be provided.

Dr. Carlos Rojas stated that in general, antimony remains the drug of choice for all forms of leishmaniasis. Nevertheless, echoing the comments of Dr. Chang, complicated distribution schemes for getting drug to the patient, results in delayed treatment which may contribute to disease complications and ultimately more difficult and costly treatment. His comments again seemed to stress the same theme as Dr. Chang is asking how can we avoid having to bring the patient to the hospital and accomplish diagnosis and treatment at the local health post. To achieve these goals there is a need for trained personnel and technology for both diagnosis and treatment. No quick, easy solutions were offered, but it was clear that a consensus was present that a priority should be to move things to the level of the rural health post.

PANEL DISCUSSION #4:

"Is Vector and Reservoir Control Possible for Leishmaniasis?"

Italo A. Sherlock¹

A review of the epidemiological changes (including control and prophylaxis) made by Ward (9) for the three decades preceding 1977, indicates that few new methods or measures for the control and prevention of the leishmaniasis, either in the Old and New World, were initiated.

The strategies and methods used to control the American leishmaniasis vary in accordance with the two general types of pathologies: visceral and tegumentary. Each type has its unique epidemiology.

American Visceral Leishmaniasis

In the epidemiology of American visceral leishmaniasis, the etiologic agent is Leishmania chagasi; the principal vector is Lutzomyia longipalpis; man and dogs are host victims but serve as the most important domestic reservoirs of the parasite. Foxes and opossums, found naturally infected in some foci, probably represent sylvatic and/or peridomestic reservoirs. Since the behaviours of these two types of mammals and their real importance as reservoirs is still unknown, they are not generally included in control measures.

The works that have been published about the control of American visceral leishmaniasis deal with three methods of control. They are: (i) treatment of human cases; (ii) elimination of infected dogs; (iii) and spraying of insecticides against the vector. In this way it is possible to achieve a notable decrease in the incidence of human cases of the disease. However, as soon as control measures are interrupted, the disease breaks out again and continues its natural history.

Treatment of human cases

According to Alencar (1) the Chinese advised that the treatment of all human cases should be the first prophylactic measure. Thus, in China, the incidence of visceral leishmaniasis has been successfully reduced. However, in the northeast of Brazil, in the treatment of human cases a low rate of incidence has been obtained, but the treatment

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of human cases alone was not sufficient to effect a substantial reduction in the incidence of American visceral leishmaniasis.

The elimination of infected dogs is the principal landmark in the control of visceral leishmaniasis. The elimination is necessary because there is no treatment for the disease in dogs. Moreover, pure bred dogs must be given special attention because they are very susceptible to infection. The immigration of dogs from endemic foci to non-infected areas must be blocked to complete control. Observations to determine if the elimination of infected dogs is sufficient to control the incidence of human cases have not been made.

Control of the vector with insecticides

Deane (3) suggested that a high density of *L. longipalpis* is strictly necessary for the incidence of human cases. This hypothesis can be supported by the existence of canine leishmaniasis in some areas of Brazil (and Texas, USA) without human cases (with or without a very low density of sandfly vectors).

Deane, Deane and Alencar (4) have evaluated the effects of DDT by observing the density of *L. longipalpis* in sprayed and unsprayed areas. Over a four month period it was verified that *L. longipalpis* almost entirely disappeared inside houses immediately after spraying and were still very rare four months later. In the shelters of domestic animals the sandfly density also fell sharply, but not as much as indoors. Three months later, the population of flies had returned to normal.

With the guidance of Alencar (1,2) the "Brazilian Campaign for the Control of Visceral Leishmaniasis" has treated 2,096 human cases, and killed 78,929 infected dogs. In 14 endemic localities which were also sprayed, the incidence of visceral leishmaniasis was reduced by 67.7%, in contrast to the 14 unsprayed control endemic localities where the rate of incidence decreased by only 25.4%. Similarly, between 1965 and 1969, using the three control measures mentioned above in the endemic area of Jacobina, Sherlock and Almeida (9) demonstrated that no human or canine cases were recorded in the last two years of the project.

Alencar emphasizes the existence of an irregular rate of incidence of visceral leishmaniasis that did not have a definitive reduction in those localities where only the human cases were treated and dogs eliminated. According to Alencar, this fact indicated that failure to spray was an inefficient method to control the disease. However, we still do not have any data or knowledge to unequivocally say if only one of the measures is sufficient to control American visceral leishmaniasis.

Tegumentary Leishmaniasis

From 1945 to 1947, Hertig and Fairchild (6) sprayed DDT with a 5% solution of kerosene in houses and on surrounding stone walls. The sandfly population was

markedly reduced for 12-19 months and new cases of "uta" disappeared. Similarly, Horror (7) reported a reduction in the rate of infection of dogs (from 56.2 to 14.7%) due to the use of insecticides by homeowners.

Guimaraes and Bustamant (8) have observed an effect of DDT on the control of malaria and the incidence of cutaneous leishmaniasis in Rio de Janeiro. The survey, carried out five years after semi-annual sprayings inside houses, showed that the foci were extinct. The incidence of human cases fell from 12.4% to 0.3%. Lutzomyia intermedia, the supposed vector, was not found inside houses but its density in the peridomestic sites continued to be the same as before spraying.

In the Amazon region, insecticides have been used on the trunks of large trees in strategic locations (such as camp sites with baths) and highly utilized paths and tracks. This was also the method employed by the "Jari Project". In spite of the rainy weather, its effect on decreasing the sandflies population remained for more than a month.

The following measures are suggested according the leishmaniasis species: Leishmania braziliensis - In forests control is not possible by means of insecticides or elimination of the sylvatic reservoirs. When the disease moves to urban or peri-urban sites some changes occur in its ecology, including changes of vectors. In the north of Brazil, the spraying of houses where human cases occurred gives some protection for healthy occupants and prevents the incidence of new cases. In middle and southern Brazil, after ecological transformation of the areas, mucocutaneous leishmaniasis begins to be transmitted inside houses by Lutzomyia intermedia and L. whitmani as well as in peri-domestic sites. In these cases, insecticidal measures give some indication of the control of transmission. In this situation, domestic reservoirs like dogs, donkeys and horses, appear to be epidemiologically important. However, studies on their elimination and their roles were not carried out.

In conclusion, I would like to emphasize that the picture of the ecology and epidemiological characteristics of the leishmaniasis in the New World remains incomplete. In spite of the research that has been carried out for decades more multidisciplinary studies are necessary to answer numerous outstanding questions. Only then will it be possible to have more efficient methods for the control of the leishmaniasis.

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Introduction

Among the leishmaniasis forms occurring in Tunisia, two are causing public health problems: (i) Zoonotic Cutaneous Leishmaniasis (ZCL) that is epidemic but relatively benign and (ii) Kala-azar (KA) that can cause deaths in the absence of treatment. Accordingly, control measures can be undertaken on 3 levels: (i) the treatment of cases; (ii) the control of vectors; and (iii) the control of reservoirs

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Treatment

Glucantime (antimoniate meglumine) is used to treat all forms of leishmaniasis. A 15-20 day treatment for KA requires hospitalization, while serious cutaneous leishmaniasis forms need only a "minute" treatment by intralesional infiltration. Presently, there is a trend to not to treat plain ZCL forms for which development is usually quick and immunizing. To avoid secondary infection, the use of local antiseptics is satisfactory.

The Control of Vectors

Residual insecticide spraying during anti-malarial campaigns in Tunisia (1968-72) resulted in a reduction of the yearly incidence of both VL and CL.

The control of vectors requires a good knowledge of the biology and bionomics of sandflies. Although the breeding places of *P. papatasi* (the vector of ZCL) and *P. perniciosus* (the probable vector of KA) are still unknown, the entomological surveys carried out in the mixed focus of ZCL and VL in the Sidi Bouzid areas, were informative.

The *P. papatasi* transmission season was shown to occur between May and November with a maximum peak density during July and August and a maximum of transmission during July and August and a maximum of transmission during September. *P. papatasi* population represented 84.6% of the total species population encountered. *L. major* MON. 25 was isolated on several occasions in different foci. The anthropophily of *P. papatasi* was confirmed by man landing catch techniques. This species was shown to be exophilic and endophilic. However, more than 60% of the *P. papatasi* population were caught in the rodent burrows; only 22.1% in human dwellings and 17.7% in animal stables.

P. perniciosus was shown to have two population density peaks, one during July and a second one during October. Approximately 58.6% of the specimens were caught in stables near dogs and 31% in human dwellings. The anthropophily of *P. perniciosus* is well established. *L. infantum* was isolated from this species in Italy and Algeria and but not yet in Tunisia.

Conclusion

Observation made about *P. papatasi* the ZCL vector, clearly show that applying insecticides intra or peridomiliary would not affect more than one fourth of females, the remaining three fourth being found rodents' burrows. Chemical control of the burrows seems difficult given the extensive areas to be treated. Only burrows destruction could be foreseen as feasible.

The control of P. perniciosus by insecticides is in theory possible in human dwellings and in stables. However, the sporadic character of the disease in humans and the absence of the stability of the human KA foci makes it difficult to implement insecticide control campaigns on a large scale essentially because of the very high cost of such measures.

Individual control measures such as individual indoor spraying, the use of nets (treated or untreated) and repellants remains difficult to apply in affected areas which are mostly poor.

Control of Reservoirs

A - Rodents

Ecology of ZCL Reservoirs

	<u>P. obesus</u>	<u>M. shawi</u>
Habitat	. Large areas of halophytic soil with chenopods . Shepp breeding areas . 1 female per burrows . Dense colonies/Km²	. Corn corps areas . Jujub - trees . Near cactus plants . Weak density/Km²
Food Requirements	. Chenopods exclusively	. Granivorous
Reproduction	. 25-36 days . All along the year	. 21 days . All along the year
Activity	. Diurnal . Weak migrations	. Nocturnal . Large Migrations
Leishmanial Lesions	. Discrete	. Inflammatory +++
Rate of Infection	. Up to 100%	. 10% to 20%
Control by Rodenticides	. Difficult . Brodifacoum - (Klerat) anti-coagulant only	. Easy . All anti-coagulants . Zn phosphide in Tunisia

Control of Rodents

	<u>P. obesus</u>	<u>M. shawi</u>
Ecological Control	. (++++) Difficult . Modifications of the environment: - Removal of chenopods - Plantations of Acacia - Working lands the "Sfax way" - Reintroduction of Camels? . Community participation . Inter-sectorial coordiantion (Agriculture)	. (+++) relatively less difficult . Destruction of Jujub-trees . Working lands the Sfax way . Community participation . Inter-sectorial coordination (Agriculture)
Control by Rodenticides	. Difficult . Brodifacoum (Klerat) (anti-coagulant only)	. Easy . All anti-coagulants . Zn-phosphide in Tunisia

B. The Dog as Reservoir of VL (KA)

Categories of dogs in Tunisia

Owned dogs: - Domestic (Accessible but very few)

- Guards in rural areas:
- . Tied dogs (100% accessible)
- . Semi stray dogs (with owners) 70% accessible, (maximum)

Ownerless dogs: - stray dogs (not accessible)

General Control measures include:

- a. Integration of action against dogs in the basic health care control program against Leishmaniasis, rabies and hydatidosis
- b. This requires accessibility to the dogs. Thus, ownless stray dogs should be eliminated.
- c. Large scale health education programs are needed to facilitate the implementation of measures to control dog populations

More specific measures might include:

1. The selective elimination of the seropositive dogs: From the experience gained in the focus of Sidi Bouzid it was impossible to serologically screen 100% of the canine population because 30% of the owned semi stray dogs were not accessible and 100% of the ownless stray dogs are not accessible for the survey. These two samples that are not accessible represent around 30% to 35% of the total canine population of the focus. The leishmanial dogs among these samples (around 10%) are not eliminated and are able to maintain transmission into the area.

In Sidi Bouzid area, it took only two transmission seasons for the prevalence of canine leishmaniasis to reach the previous rate found at the beginning of the control. Among the other causes for failure, was the absence of studies on the ecology of dogs in the area and especially on the turnover of the canine population. Moreover, the selective elimination of owned dogs (supposedly vaccinated against rabies) could contribute to an imbalance in favour of uncontrolled and unvaccinated dogs.

2. The massive killing of dogs can be conceived only in the case of epidemic outbreaks. Combined with patients' treatment and insecticide intradomiciliary aspersion, this approach was rather successful in China. However, a Kala azar recurrence occurred in China in 1968-1975 after a relaxation of such measures. The ecological consequences of a dog massive killing are still to be assessed.

3. Treatment of Dogs: Dogs' treatment is usually made with pentamidine (Iomidine). This treatment is reserved for dogs "precious" to their owner, and must be repeated yearly since it treats the animal but does not cure it. Such treatment is used in towns. It is unconceivable and unachievable (cost, awareness), at the individual level in the rural area. In Tunisia, antimoniate meglumine (glucantime) is especially kept for human use. Using glucantime on dogs in Italy entailed the appearance of canine strains resistance of antimony, and therefore, became permanent leishmania reservoirs.

Bruno L. Travi³

Theoretically, leishmaniasis control could be targeted to one or more of the three components of the transmission cycle, the reservoir, the vector or the so-called accidental host, the human being. The complexity of the transmission cycle suggests that concentrating on a single target or utilizing only one control strategy will fail to achieve satisfactory results. It is possible that in the Old World ecological situations allow for successful interventions, but in Latin America several factors converge to create new epidemiological pictures. These factors are usually related to migratory population movements toward unexploited territories, land modification due to lumbering, and

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primitive agriculture in secondary forests, all of which result in the settlement of new communities in areas of high risk for leishmaniasis infection.

Leishmaniasis control in the New World still remains a challenge for parasitologists, epidemiologists, and health authorities. New vectors and putative reservoirs appear in the literature in increasing numbers, but this information does not seem to trigger control efforts in the endemic countries. It is possible that lack of governmental commitment has been more important than biological constraints in impeding successful control of the leishmaniasis.

RESERVOIR CONTROL

Incrimination of wild reservoirs

An increasing number of mammals of different orders have been identified as putative reservoirs of *Leishmania*, based on the isolation of amastigotes from their tissues (Dedet et al. 1989; Shaw 1988; Lainson et al. 1989). They are considered the natural source of infection for zoophilic sandflies and for those species that would alternately feed on man and animals according to circumstances. Theories on their participation in the transmission cycle encompass behavioural aspects responsible for territorial overlapping with human habitations and activities, as well as highly efficient host-parasite associations in which infected sandflies transfer leishmania from primary or secondary forests to peridomestic and domestic settings.

When evaluating potential reservoirs, little attention has been paid to the distribution and density of mammals within an endemic focus. No quantitative observations are available regarding sandfly-mammal associations, dispersal, and prevalence of infection of adult female sandflies arising from specific animal dwellings. Xenodiagnosis has proven disappointing in most cases, both with colonized and feral sandflies, and for this reason it is difficult to comprehend how some *Leishmania* species are so efficiently transmitted to the human population. In fact, with few exceptions (Rojas et al. 1990), xenodiagnosis has proven unsatisfactory to assess both vector competency and reservoir status. It seems that under natural conditions, *Leishmania* (*Viannia*) spp. infected tissues contain a relatively small number of amastigotes, making parasite intake during sandfly feeding less frequent than one would expect.

We were unsuccessful in trying to infect *Lu. trapidoi* and *Lu. gomezi* by allowing them to engorge on the lesion border of a naturally *L. braziliensis*-infected dog, as well as colonized *Lu. longipalpis* on lesions of *L. panamensis*-infected patients. On the other hand, experimentally infected hamsters are good sources for sandfly infection either with *L. panamensis* or *L. braziliensis*. Since hamsters are usually inoculated with 1-5 million promastigotes, it is reasonable to assume that the heavy load of parasites within the tissues offered to the vectors accounts for the positive results.

Recovery of amastigotes from the tissues of putative reservoirs varies according to the different Leishmania species, and endemic foci. With the exception of one sloth infected with L. panamensis, we were unable to recover Leishmania from a wide range of mammals during epidemiological studies in the Colombian Pacific coast where L. panamensis and L. braziliensis are endemic. The methods which proved to be ineffective for screening wild fauna, were similar to those regularly used in the laboratory to recover amastigotes from human biopsies and samples from experimentally infected laboratory animals. Recent studies in our laboratory (Martinez et al. 1991) have shown that dilution of tissue triturates resulted in higher recovery rates of amastigotes as compared to undiluted samples. This phenomenon is even more noticeable during the chronic stages of the disease when parasite loads diminish. If these observations are extrapolated to the reservoir situation, it would be reasonable to assume that amastigotes may be scarce, and for this reason they may go undetected when conventional techniques are used for parasite isolation.

Incrimination of domestic Reservoirs

Not too many years ago, dogs were the only domestic animals widely accepted as a source of infection. While little doubt was left regarding their importance as a visceral leishmaniasis reservoir, other domestic animals which have been reported infected with different species of Leishmania were disregarded as possible links of the transmission cycle. As previously mentioned, it is possible that the lack of experimental confirmation through xenodiagnosis or recovery of amastigotes from typical lesions accounted for these assumptions. In addition to the dog, donkeys and horses, in which overt disease was reported in Venezuela and Brazil (Aguilar 1990) traverse together with man vector resting sites during daytime, and share the peridomestic environment with humans when sandfly activity is at its peak. This proximity and interaction give multiple opportunities both to humans and animals to participate either as reservoirs or victims of the disease. In certain endemic foci, horses may act as important attractants of sandflies. In the province of Salta, Argentina, horses are frequently bitten in the pubic area, around the eyes, nostrils and inside the ears by Lutzomyia intermedia, the putative vector of L. braziliensis in that focus. We have experienced a marked increase in sandfly biting when collections were made close to horses, as compared with regular protected human-bait catches.

Observations in which the same vector and Leishmania species are responsible for producing overt disease, both in human and domestic animals suggests that the latter could be of greater importance than originally proposed.

Problems related with Reservoir Control

When wild mammalian fauna is considered as the target for control several constraints emerge:

1. In the population dynamics of mammals, a natural replacement occurs whenever the home range occupied by a particular individual becomes empty. This implies that trapping would result in the influx of naive specimens of the same species, which in the best of cases will become infected within a few months if they do not harbor Leishmania already.
2. While marsupials and rodents are easy to trap, other species may be difficult, and would require expertise and commitment, which are hard to obtain in most endemic areas. Certain mammals such as foxes, monkeys, kinkajous, and sloths could be endangered species for which elimination would be forbidden; relocation of wild fauna is an even greater problem that should not be considered as a feasible alternative.
3. The main question that conservationists will ask is if control measures targeted against feral animals are based on quantitative analyses, and epidemiological information as to assure a level of positive impact on health that would outweigh ecological damage. At present, these inquiries would be poorly answered.

For these reasons, wild reservoir control should be considered as an impractical approach to diminish or stop Leishmania transmission in the New World.

Although transmission dynamics studies involving domestic animals are still pending, they should be treated as potential sources of parasites. Elimination of dogs is unaccepted by most pet owners, and this approach could interfere with other control strategies that may require community participation. On the other hand, dogs are immediately replaced by naive individuals which, in the case of L. chagasi, are infected within a few months. Two alternatives are envisaged as the most viable options: a) avoiding vector-host contact by the use of repellent-insecticide collars (dogs), and harnesses or mesh covers (horses, donkeys) at the peak of sandfly activity, and b) immunological protection through the development of an effective L. chagasi vaccine. The first approach would require previous testing of the collars in the laboratory, subsequent field studies using groups of naive animals, and serological screenings for demonstrating differences in infection rates between test and control animals. The vaccination strategy has been considered by several groups of researchers and it is expected that information will be available soon in this regard. Although vaccines may be efficient in protecting dogs, evaluations should focus on how this immunological strategy affects L. chagasi infection rates in the human population. Although not proven, protection of domestic animals against L. (Viannia) spp. infections, as well as early diagnosis and treatment of humans, may contribute to diminish tegumentary leishmaniasis in certain foci.

Incrimination of Insect Vectors

Leishmania vectors are considered as such when several criteria are met. Probably the most important are: a) the identification of natural infections with the same

parasite species which is regularly isolated from the human population of the endemic focus; and b) high density of specimens at certain times of the year, and anthropophilic behavior, a combination of factors that will assure frequent vector-host interaction. Experimental studies by Warburg et al. (1991) showed that sandflies which are not involved in transmission of Leishmania could be readily infected under artificial conditions, suggesting that other factors such as ecology and sandfly behaviour are key elements for disease transmission.

Once the putative vectors have been identified and their population dynamics, and biting behaviour has been studied, strategies could be drawn either to avoid vector-man contact or reduce populations below critical levels. Albeit not systematically evaluated, sandfly control in the New World has been considered as a feasible strategy for reducing leishmaniasis in endemic foci. While for people exposed for short periods of time leishmaniasis transmission could be prevented with repellents, other approaches should be taken into consideration when disease transmission needs to be interrupted in endemic areas. Protection of men entering leishmaniasis areas for short periods of time have been achieved with jackets impregnated in n,n-diethyl-m-toluamide (DEET) (Schreck et al. 1982). Similarly, Alexander and Rojas (1991) have observed significant protection against colonized Lu. longipalpis for up to 8 hours with a repellent soap (20% DEET, 0.5% permethrin) that other research groups had tested successfully against different species of mosquitoes.

Although it is widely accepted that the zoonotic nature of the disease precludes its eradication, focusing sandfly control on those areas where man-vector contact is frequent may yield epidemiologically sound results. Farming is the most common activity related with Leishmania infection. Land is distributed in relatively small parcels, where cacao, banana and plantain are intermingled with secondary forest; coffee plantations in the mountainous regions are protected from the sun by large trees. Sandfly populations in these areas are composed of species that were capable of adapting to modified environments and perpetuate the anthroponosis. Since it has been demonstrated that there is a dose-response relationship in the risk factor for infection, it is clear that reducing the number of sandflies at the working sites would be highly beneficial. Plantations in the tropics are small when they are owned by local settlers, and thus their forest activities are restricted to limited areas. In these places both mechanical modification of the sandfly resting places, and chemical intervention targeted to specific microhabitats could be attempted. In general, adult sandflies seem to be susceptible to commercially available insecticides. Although in some areas resting places are difficult to identify, chemical spraying could be directed tentatively towards the buttresses and holes of the largest trees. DDT spraying has been associated with a marked decrease in sandfly activity and incidence of visceral leishmaniasis cases in Bangladesh (Elias et al. 1989), and was used in Brazil against Lu. longipalpis (Deane et al. 1955) and Lu. umbratilis (Ready et al. 1985). At the present time, this compound is regarded as an environmentally unsafe insecticide which could be replaced with other safer compounds. In fact, due to the lower toxicity for man and animals, Malathion and photo-stable pyrethroids have been considered for this kind of intervention.

As part of ongoing control studies, we have tested several insecticides against both adult and immature stages of sandflies. Colonized Lu. longipalpis were more resistant than feral Lutzomyia spp. collected in a botanical garden. Test papers impregnated with a 1500 ppm concentration of Deltamethrin (K-othrin S 25^R, Roussel Uclaf) produced 95 and 100% mortality (Abbott) in Lu. longipalpis and Lutzomyia spp., respectively, when evaluated at 24 hours following a 10 minute contact exposure with the compound. To achieve similar results Malathion and to be used at concentrations higher than 3000 ppm. These experimental results suggest that Deltamethrin, due to its high potency and low toxicity, could be the first choice for wall spraying inside houses in foci with intradomiciliary biting. Falcao (1988) reported that the number of Lutzomyia spp. declined for almost 1 year after intra- and peri-domicile spraying of 25 mg/m² of Deltamethrin in a leishmaniasis focus of Viana, Spirito Santo, Brazil. Some preliminary field studies by Le Pont et al. (1989) in a sub-Andean village of Yungas, Bolivia suggested that deltamethrin sprayed at the beginning of the rainy season at the domicile and peri-domicile settings could reduce the number of Lu. nuneztovari anglesi, were not clearly affected by this intervention. These observations stress the importance of studying vector population dynamics before implementing control strategies. Insecticides may kill insects that have acquired the infection in the forest, and thus control measures targeted to the domicile or peridomicile may not have any effect on transmission. The opposite would happen with species which are infected close to or in domestic settings. Mark-recapture techniques are needed to understand dispersal of sandflies in different foci of transmission, and to optimize insecticide spraying.

CONCLUSIONS

When targeting control measures towards vectors and reservoirs it is important to consider that transmission may take place at three different sites:

a) **Extradomicile:** transmission is related mainly with agricultural activities, which are usually carried out in specific places (small parcels of land) that could be modified by mechanical intervention such as clearing the forest, thus reducing appropriate environments for sandflies. Vector densities could be lowered by selective spraying of large trees and other resting places. Other forest activities that are not performed continuously in the same area or may be sporadic, such as lumbering or hunting, should take advantage of personal protection with clothing and repellents. Reservoir control is not indicated.

b) **Peridomicile:** this setting is shared by man and domestic animals during the peak of sandfly activity facilitating alternate feeding of insect vectors. Spraying of animal shelters has proven effective, and since elimination of pets or work animals is unacceptable, the utilization of protective collars or harnesses impregnated with repellents or pyrethroids should be explored.

c. **Domicile:** according to preliminary information, wall spraying is an efficient methods for reducing sandflies indoors. Further, it is possible that pyrethroid impregnated bednets and/or curtains could yield significant protection. Cost-benefit evaluations in different endemic foci should decide which of these approaches will be more beneficial in each particular case.

Assuming that well designated studies would confirm what isolated control trials have suggested, a greater challenge beyond the biological viewpoint awaits: implementing recommended control programs involving community participation and Public Health authorities, with the aim of assuring effectiveness and long-term commitment.

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DISCUSSION SUMMARY

With respect to visceral leishmaniasis Dr. Sherlock advocated detection and elimination of infected dogs. He also felt that peri-domiciliary control, but not sylvatic control of vectors was achievable. Dr. Ben Rachid commented that in devising strategies for achieving vector control, we need to account for different periods of activity of different sandfly species. He indicated that vector control is difficult because of cost and because of the extent of the areas to be treated. In comments that echoed those of Dr. Sherlock he stated that achieving vector control is more realistic in areas where peri-domestic transmission takes place. He also indicated that whereas control of rodents reservoirs may be possible there were significant problems with the elimination canine reservoirs.

Dr. Travi stressed the importance of confirming implicated reservoirs and that techniques for reservoir identification need to be improved. He went on to say that vector and reservoir control strategy needs to be feasible. He discussed the limitations of reservoir control including natural replacement, problems with domestic animals, endangered species. In general, he did not believe that reservoir control was a practical strategy to disease control. With respect to vector control, current insecticide application schemes are only transiently effective and do not evenly eliminate all sandfly species. Dr. Travi stressed what he believed to be the important need to evaluate larvicidal vs adulticidal effects of insecticides. He also cautioned about results of research obtained with using lab reared vs. wild type flies. There was substantial lack of agreement amongst participants, with comments both pro and con, regarding the likely effectiveness of achieving control with larvicidal agents. It would seem that more research is needed to clarify this point. In closing Dr. Travi commented that wild reservoirs cannot be controlled in the new World and that domestic reservoirs should be

protected flies where this is possible. Furthermore, efforts should be directed at vectors, extra-domiciliary, peri and domiciliary insecticide application, with research efforts directed at optimization of approaches.

Dr. Perez emphasized the need not to ignore and in fact to stress personal protection measures. Several individuals including Drs. Arias, Ward and Desjeux stressed that DDT was perhaps the most effective insecticide available for vector control and that its utility has been overlooked because of concerns about toxicity. It was argued that in the concentrations needed to achieve control of sandflies that is did not represent a hazard. In particular, the large doses used in agriculture have given it a bad name, but in the low doses required for domestic application it is safe, cheap and effective. Dr. Desjeux raised the question of DDT resistance, and this needed to be considered in specific situations, although he did not believe that this was a widespread problem. Dr. Chang raised the concern that funding agencies forcefully discourage the use of DDT and it may be important to get this issue reconsidered at the levels of both funding agencies and governments.

Dr. Mutinga stressed the need for community education to modify cultural practices that promote the development of peri-domiciliary breeding grounds for flies. This should be done as an important adjunct to or in lieu of insecticides where the latter are not practical.

PANEL DISCUSSION #5:

"Immunological Considerations in the Control of Leishmaniasis"

Immunological Considerations in the Control of American Tegumentary Leishmaniasis caused by Leishmania of the subgenus Viannia

By

N.G. Saravia¹

1. Does natural infection confer resistance to new infection and disease? Are individuals who have had and recovered from leishmaniasis likely to experience leishmaniasis again?

The frequent presence of scars typical of prior leishmaniasis in patients consulting for the diagnosis and treatment of active lesions and the documentation of recurrent lesions due to the same of distinct parasites in prospectively evaluated patients, indicate that natural infection with leishmania of the subgenus Viannia does not necessarily confer resistance. In fact, longitudinal assessment of the Pacific Coast of Colombia, indicated that individuals with evidence of prior leishmaniasis had a higher relative risk of new disease than did naive individuals. Continual exposure to infection and other factors associated with living in an endemic area may contribute to this pattern of recurrent disease. To our knowledge, recurrence is rare among individuals who have acquired leishmaniasis during temporary exposure to transmission, i.e. soldiers, laboratory workers. Requirements for immunoprophylaxis may therefore differ for individuals permanently residing in endemic areas and for those transitorily exposed.

2. Does infection with a particular Leishmania induce cross-protection to other Leishmania?

Considerable heterogeneity has been observed among Leishmania of the Viannia subgenus. Variation is phenotypic as well as genotypic, and evidence for antigenic heterogeneity has been provided by typing analyses utilizing monoclonal antibodies. Since experimental animals vary in their susceptibility to different Leishmania, and the subgenus Viannia (braziliensis complex) is notoriously of limited pathogenicity in most animal models, cross-immunity as evidence by diminished pathology, is not a simple matter to evaluate. Nevertheless, our experience with human infection indicates that individuals who have had disease

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due to *L. (v) panamensis* remain susceptible to re-infection with closely related organisms (distinct zymodeme or schizodeme) of the same taxon as well as to *L. (v) braziliensis* (Saravia et al. 1990. Lancet Vol. 336: 398-402). Re-activation of infection and the appearance of new lesions following resolution of disease with or without specific treatment, suggests that even immunity acquired to the homologous organism may not protect against further disease. It will be pertinent to determine the risk factors for recurrent disease so that appropriate control measures may be targeted to the prevention of recurrence.

There is increasing evidence for the latent persistence of *Leishmania* infection. Since subclinical infections probably outnumber clinically apparent infections, it will be important to define the potential contribution of subclinical infection to herd immunity, as a source of further transmission, and future disease.

Exogenous re-infection and the incidence of disease in individuals having a prior positive skin test argues against the notion of premunition as a mechanism of protection in American tegumentary leishmaniasis due to the *viannia* subgenus.

3. Who is susceptible to leishmaniasis? How can susceptible individuals be identified?

Lack of understanding of the natural history of human leishmaniasis restricts our ability to precisely define susceptibility, particularly in terms of disease. An operational definition of susceptibility based on the absence of immunological evidence of prior exposure to *Leishmania* may provide a reasonable approximation of susceptibles with respect to infection. Although further investigation is needed in order to determine whether individuals with well-defined clinical "phenotypes" reflect intrinsic host differences in disease susceptibility, chronic and/or recurrent disease may provide an indicator of susceptible individuals.

4. Does the immune response participate in the pathogenesis of tegumentary leishmaniasis?

Direct evidence for the participation of T lymphocytes in the progression of disease had been obtained in the inbred mouse model of tegumentary leishmaniasis caused by *Leishmania major*. Phenotypically distinct subpopulations of T lymphocytes have been shown to adoptively transfer either protective or disease enhancing response to *L. major*. In human infection with *Leishmania* of the *viannia* subgenus, non-resolving, chronic disease is associated with immunologic hyper-reactivity. Likewise, mucosal disease is associated with increased cellular and antibody responses to *Leishmania* antigens. These patterns of response provide evidence, albeit indirect, for the participation of the immune response in the pathogenesis of human tegumentary leishmaniasis.

5. **Can a non-healing or non-protective response be converted to a healing or protective response? How? Which antigens, preparation, route?**

Elegant experiments by Howard, Hale and Liew established that the healer and non-healer phenotype of *L. major* infection in inbred mice is determined by hematopoietic cells and that the selective ablation of radiation sensitive cell populations could convert the non-healer to a healer phenotype. Other investigators have subsequently shown that a variety of immunomodulatory treatments can also reverse the non-resolving progression of *L. major* infection in the highly susceptible BALB/c strain. The immunotherapy experience of investigators of the Instituto de Biomedicina in Venezuela led by Dr. Jacinto Convit, (Convit et al., 1987. Lancet Vol 1:401-404) indicates that a healing response can be induced in patients infected with *Leishmania* of the *viannia* subgenus by the administration of leishmanin and BCG. Even chronic lesions characterized by a hyperreactive cutaneous response to *Leishmania* antigens were shown to heal following this immunologic "perturbation" of a non-resolving response to *Leishmania* infection. While it remains to be determined whether immunotherapy results in resistance to new disease, it is clear that the untoward effects of the immune response can be reversed to the benefit of affected patients. This intervention may be optimized by the utilization of defined antigens and alternative adjuvants.

6. **How can acquired resistance be assessed? Which immune response parameter correlates with protection?**

Assessment of resistance is precluded by an understanding of the pathogenesis of leishmaniasis as well as the immunologic basis of resistance to disease. Neither cell-mediated nor antibody responses to whole antigen preparations are correlated with resistance, i.e. protection from new infection and disease in man. Since protective and exacerbative T lymphocyte subpopulations are preferentially induced by distinct antigen fractions (Scott et al. 1988. J. Exp. Med. 168:1675-1684) resistance may correlate with the qualitative response to defined antigens or even epitopes. In any case the identification of immunologic correlates of resistance is a priority concern since it would allow populations to be classified as susceptible or resistant and vaccine efficacy to be expeditiously evaluated.

7. **What exactly would we hope to prevent by immunoprophylaxis? Chronic disease, any disease?**

If individuals who have already acquired infection contribute to the disease burden in endemic areas, alternative control measures will be needed to prevent the progression of latent infection to disease. Are or can humans be reservoirs or "carriers" of infection? If so could immunization prevent them from becoming reservoirs?

The prevention of severe disease alone may justify vaccination. If all or most disease cannot be prevented, length of treatment might conceivably be shortened for lesions developing in immunized individuals. Reasonable and achievable goals should be set for the immunoprophylaxis of New and Old world leishmaniasis in accordance with the disease burden and natural history of the parasites involved.

Immunological Considerations in the Control of Leishmaniasis

By

F.J. Andrade-Narvaez, G. Valencia-Pacheco; M.R. García-Miss

Introduction

There is a great volume of information regarding immunological studies of the Leishmaniasis, including clinical and experimental, ranging from applied research such as development of new diagnostic tools up to very basic research on immunogenetics and molecular biology studies. For the last 10 years excellent reviews of this field have been done (1-4). However, examination of the role of immunological consideration in the control of the Leishmaniasis has been rare.

Objectives

On this basis, it has been considered necessary to carry out a review of our experience studying Mexican Localized Cutaneous Leishmaniasis (Mex LCL), well known as "Chiclero's Ulcer" (5). The immunological aspects incorporated into our Leishmaniasis Programme have the following objectives:

- To develop new immunological diagnostic tools.
- To identify host risk factors associated with clinical outcome.
- To study host-parasite relationships.
- To characterize the immune response.
- To develop vaccine "candidates".

State of the Art

Development of new immunological diagnostic tools: The first task was to develop an enzyme-linked immune assay (ELISA test) to detect IgG antibodies to L. mex. mex. determining its sensitivity and specificity. A total of 223 human sera were assayed and results obtained, including "chronic" cases (low positivity of 54%), which are relevant due to the fact that they are useful to support the diagnosis of Mex LCL. Serodiagnosis by ELISA has been positive in the study of Leishmaniasis both in the Old World (6-9) and in the New World (10-12). This preliminary study proved to be useful as a contributory diagnosis method in cases of suspected Mex LCL (13).

The knowledge regarding IgM antibodies against leishmania sp is very poor and Mex. LCL is not an exception. Therefore, we decided to develop an ELISA to detect IgM antibodies against promastigotes of L. mex. mex. according to established methodology (14) with a simple modification; all sera were treated with 2-mercaptoethanol (15). The results have confirmed the presence of IgM anti-Leishmania mex. mex. and are part of the second phase of the IDRC-supported project.

The ELISA for IgM and IgG anti-Leishmania mex. mex. developed in our studies show that: i) IgG anti-Leishmania mex. mex. ELISA has a sensitivity of 76% (91% in "acute" cases and 54% in "chronic" cases, criterion arbitrarily established by Biagi since 1953) and specificity of 93%; ii) IgM anti-Leishmania mex. mex. ELISA has a sensitivity of 56% and specificity of 92%; iii) There is a significant association of both IgG and IgM positivity with a clinical evolution lesser than 6 months and presence of "disorganized granuloma" in the histopathological image (17).

Identification of host risk factors associated with clinical outcome: We have studied a total of 179 "well defined" (by TDR/WHO/LEISH) cases of Mex. LCL that included complete clinical history, biopsy, parasitological and immunological studies. General characteristics are as shown in Table 1.

Preliminary statistical analysis has shown important and interesting associations or relationships which are presented in Table 2. Although this study is not complete, the results can be considered important in terms of immunological considerations of Mex. LCL control:

- (i) identification of "high risk" groups which can be targetted for control strategies;
- (ii) a predominant clinical picture supporting Mex. localized CL (no metastasis, no lymphangitis and/or adenopathy) which is extremely important to support characterization of local immune response at skin level;
- (iii) regarding the clinical picture, there is a change in historical concepts such as "Chiclero's Ulcer of the Ear" (Seidelín nomenclature) to be analysed and "chronic" percentage was 10% less than expected;
- (iv) the significant associations shown in Table 2 among certain clinical, histopathological images, including presence/absence of parasite and antibody IgG anti-Leish. mex. mex. positivity, when taken into account lead us to believe the presence of varied clinical patterns and their subsequent evolution. Therefore, the necessity to review "classifications" previously established in the literature. It is quite clear that there are certain findings which could be organized as criteria to suggest as "acute",

**Table 1: Mexican Localized Cutaneous Leishmaniasis (Chiclero's Ulcer)
Host Risk Factors Associated with Clinical Evolution**

n= 179 (clinical history, biopsy, parasitology and immunological studies)

Sex: Male 148 (82.6%); Females 31 (17.3%)

Age: 10-44 years (128/148 or 86.5%)

Clinical Picture (predominant):

	<u>No.</u>	<u>Percentage</u>
Type: Ulcer	156	87.5%
Area (cm ²) <6	143	90.0%
No. Lesions: single	134	74.8%
Location: Ear	44	24.5%
Limbs	82	45.8%
T. Evolution: < 6 months	143	80.0%
6-12 months	20	11.17%
> 12 months	16	8.93%

**Table 2: Mexican Localized Cutaneous Leishmaniasis (Chiclero's Ulcer)
Clinical-Histopathological-Immunological Relationships**

Test	Response	Correlation (X ²)
1. ELISA IgG+	T. Evolution < 1 month	6.06
2. ELISA IgG+	Parasite positive biopsy	9.17
3. Parasite positive	T. Evolution < 6 months	11.76
4. Parasite positive	Disorganized granuloma	5.01
5. Parasite negative	Organized Granuloma	6.16

cases with an evolution less than 6 months ELISA IgG and IgM positive, parasite demonstration commonly positive and presence of "disorganized granuloma" in the biopsy;

(v) from studies done to date it has been possible to identify at least five patterns: "acute" with spontaneous healing; "acute" responding to specific treatment, "chronic", "reinfected", and "recurrence".

PROPOSED FURTHER IMMUNOLOGICAL STUDIES (1992-)

Antigen identification employing specific antibodies: One approach to be initiated will be the identification of specific antigens of *Leishmania mex. mex.* employing sera from patients IgG and IgM ELISA positive. We have the advantage of parasites isolated from each patient and its serum. Through this approach we expect to improve and increase sensitivity and specificity of ELISA's and develop a field serodiagnosis test.

Characterization of immunity in human cutaneous leishmaniasis: This is a long-term study to be initiated in January 1992 and its ultimate goal will be to obtain purified or recombinant. Vaccine candidates.

Vaccine approach: As it has been stated since the beginning of Immunology, the main objective is to apply and/or develop vaccine "candidates" have been tested. However, it has not been open to discussion, and the possibility to extend or reproduce some preliminary results obtained to other geographic areas with Cutaneous Leishmaniasis remains to be done.

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DISCUSSION SUMMARY

This was an interesting session in which Drs. Saravia and Andrade raised numerous provocative questions about immunity to leishmania and possible implications for disease control. Dr. Saravia stressed the important point that within in endemic areas, only a minor fraction of the population at risk for infection will develop disease. It will be important to develop tools to identify these patients which would permit targeted control strategies. Unfortunately, while this entire discussion did provide much interesting food for thought, it raised more questions than it provided answers. Knowledge in this area at this time is such that no practical recommendations for disease control can be offered. More basic and clinical research is needed in this area before immunological approaches to disease control can be anticipated. There was a consensus that ultimately a vaccine approach should succeed.

Working Group #1: The Role of the Community in Leishmaniasis Prevention and Control

This working group consisted of representatives of Ministries of Health, non-governmental organizations involved in community based health research and interventions, medical personnel, epidemiologists, anthropologists, sociologists, representatives of funding agencies, and an entomologist. The discussion was lively, often heated, and often (so it seemed at the time) unproductive. In the end, however, the group came to a common understanding of the problem of involving communities in disease control programs, and produced two summary tables which we felt could usefully guide both neophytes and experts, whether in community participation or leishmaniasis control, in thinking about and actually involving communities and local health workers in this process.

The lively, heated, and at times stalemated debate is likely to be repeated in any such endeavour; emphasizing that there are no simple, fool-proof recipes. However, the suggestions that the group members made arose from actual experience. Village health workers and families of patients can and have successfully taken the major responsibility for case detection, administration of medication, and follow-up of this complex set of diseases, where effective lines of referral have been in place. When the tables (attached) speak of collaboration between the community, the health system (both technical and political aspects at multiple levels), and researchers (in natural, health and social sciences), we mean this in a literal but dynamic sense; different actors will play more or less prominent roles at various times, both within a single "program" as well as in the different social, cultural, political, ecologic and medical contexts in which the leishmaniasis occur.

The discussion centred around two problems and their intersection: first, what is "community participation", and how can it be encouraged, sustained, and evaluated; and second, what is the importance of leishmaniasis in a given context, and what are the elements or components necessary for its prevention and control. The experience of each of the group members was critical in addressing each problem in its own right and especially to develop an approach for integrating the two that would do justice to the complexity of the problem while also allowing something to be done about it. The recommendations reflect both the process and the content of the discussion, and are summarized in Tables 1 and 2.

Table 1 is a series of questions to guide the "planner" who wishes to involve communities in any disease control program, in this case leishmaniasis. The questions address two issues:

- a) defining the terms and terms of reference as clearly as possible;
- b) undertaking a situation analysis of the health issue in question, as well as of the community and its context.

Table 2 proposes a dynamic, multi-faceted process of collaboration among the various actors who are performance affected by a health problem and its control or prevention. It is organized around the components necessary for control of this kind of disease: **research, case management, surveillance, prevention** (at reservoir, host, vector and ecosystem levels), and - really the synthesis of all of the above - **program planning, development, and evaluation.**

Community members can be involved in each of these components, as can researchers, health care providers, and politicians. The table suggests that in many cases the initial leadership will come either from scientists or the health care system, but at times the community itself will initiate fairly comprehensive control strategies. This has been the case, for example, with AELO - the Asociacion de Enfermos de Leishmaniasis de Ocongate - a self-help group in the Peruvian Andes. The desired outcome is not that "communities" eventually bear the responsibility and cost of everything from research to supplies, but rather that the potential of the community to identify important problems and possible solutions and to be involved in decision making at all stages be recognized and incorporated in integrated strategies of health care.

Questions of financing, referral, supervision, and reasonably reliable and effective functioning of the primary health care system must be addressed. Although at times "community participation" is seen as a way of absolving governments of the responsibility (and cost) of maintaining an equitable health care system, the working group did not share this perspective. Rather, we felt that what is often considered to be highly "sophisticated" health care and disease control can be effectively decentralized to the community level, but only with the participation and involvement of other levels of the health care system and society in general. While longterm costs may well be decreased and effectiveness increased if communities are actively involved, an initial and ongoing investment of time and money cannot be avoided.

Table 1: Community Participation in Leishmaniasis Control: A Series of Questions to Guide the "Planner"

1. <u>WHAT DO YOU MEAN BY "COMMUNITY"?</u> - there are many potential definitions, for example: · a specific settlement, town, or village; · patients or sub-groups of patients (e.g. self-help groups) · a high risk group (eg, migrant workers) - heterogeneity present in all communities, thus many potential roles
2. <u>WHAT DO YOU MEAN BY "COMMUNITY PARTICIPATION"?</u> - see, for example, the work of Susan Rifkin - we mean community involvement at all stages, from problem definition and formulation, to baseline research, to development and implementation of interventions, to evaluation and monitoring - we do not mean paying for services, or "contributing" labour, or "participating in what I tell you to do"
3. <u>WHY COMMUNITY PARTICIPATION?</u> - clarify what you and other interested parties hope to achieve through community participation - reasons for promoting it range from moral commitment, to attempts to decrease one party's costs - a pragmatic reason for community involvement is that those affected are in a good position to advise on what is (un)likely to work, and that their cooperation is required to address a complex disease not easily controlled by the health system alone, especially with limited resources
4. <u>HOW IMPORTANT A PUBLIC HEALTH PROBLEM IS LEISHMANIASIS HERE?</u> - consider current health and socio-economic impact of the disease, and the potential impact of interventions (e.g. severity and avoidability of risk factors such as forestry or migration) - can leishmaniasis control be integrated with other locally and externally identified health or social problems?
5. <u>WHAT KIND OF HEALTH CARE IS AVAILABLE HERE?</u> - formal health care system: lines of supply, referral, and supervision are crucial for decentralizing disease control to peripheral health centres and villages - informal and traditional systems: consider possible links, from household care to self-help groups to traditional healers
6. <u>WHAT IS THE HISTORY OF COMMUNITY INVOLVEMENT IN HEALTH HERE?</u> - not all communities are eager to participate in leishmaniasis control! negotiation will be necessary - many models have been tested - a literature search of your area will give a sense of the possible timeframe - timeframe usually medium to long; occasionally short for a one-shot campaign

Table 2: Community, Health System, and Researcher Collaboration in Leishmaniasis Control: Suggestions for entry points, processes, and supervision based on existing experience with community involvement in leishmaniasis control

COMPONENTS OF CONTROL	WHO CAN DO WHAT, WHEN, AND UNDER WHAT CONDITIONS
RESEARCH/ COMMUNITY DIAGNOSIS	- basis for planning, implementation, monitoring a) Baseline community diagnosis by multidisciplinary research team with community and health system collaboration (info on demographics, socioeconomic and ecological situation, risk activities, underlying socio-cultural determinants) b) Ongoing, focused research by community members and health care providers with researcher collaboration (to monitor and adjust action)
CASE FINDING (DIAGNOSIS), TREATMENT, AND FOLLOW-UP	- start this component together with community diagnosis. - initiated by health workers through promotion of services, education, appropriation of biotechnology - eventually, much case management can be decentralized to peripheral health centres and villages, where health promoters, patients and community groups share passive and active case-finding, and treatment and follow-up of appropriate cases - THIS NEEDS A CLEAR AND FUNCTIONAL REFERRAL SYSTEM, BUT CAN BE ADAPTED TO LOCAL SITUATIONS - communities key in identifying and co-managing social, psychological and economic costs of illness - financial burden shared by state, employers, donors, community, and patients

COMPONENTS OF CONTROL	WHO CAN DO WHAT, WHEN, AND UNDER WHAT CONDITIONS
SURVEILLANCE	- surveillance can meet range of needs - local to international. Locally relevant information more likely to be sustainably collected - INFORMATION MUST FLOW BOTH WAYS IN A TIMELY AND USEFUL FASHION - initially - passive surveillance, largely by health workers - increasingly active surveillance if linked with case management and ongoing community-based research - community leaders (eg teachers), patients and others can be involved
PRIMARY PREVENTION	- should address each of reservoir, host and vector, with variable emphasis according to local situation and resources - health workers key in suggesting options for protection and control, in dialogue with "experts" - community input key in adapting suggestions to local situation and testing/evaluating interventions - individual measures and local vector/habitat control may be possible at household level with health system supervision - cost sharing as under case finding/treatment/follow-up - community key in identification and potential modification of social and economic situations that force people to be at risk (political primary prevention)
PROGRAM PLANNING, DEVELOPMENT & EVALUATION	- ongoing process incorporating each of the above components, with mutual feedback among components and collaborators - can be very flexible and participatory, as different components may be appropriate entry points for both control and community participation (see above and Table 1), BUT requires ongoing commitment from all collaborators, good records, and reliable support from other parts of the system (health, finance, transport, etc)

CAUTION!!!! THIS IS A LONGTERM PROCESS, AND NOT A MAGIC BULLET!!

Working Group #2: Technology Needs, Availability and Transfer for Leishmaniasis

Charged with the task of examining technology needs, availability and transfer for leishmaniasis, the working group began its session by brainstorming and compiling a "wish list" of technologies for leishmaniasis. Once all the ideas were on the table the working group then went on to screen this initial list. For each technology discussed the group asked the following questions:

- Is this technology realistic?
- What is the timeframe for development?
- What are the cost implications associated with the technology?

Through this process of elimination, the group was able to focus its attention on those technologies for leishmaniasis that are (i) most needed and (ii) feasible both in terms of time for development and cost. Considerable discussion was also devoted to identifying the prospective use and users (applicability) of each selected technology. Table 1 summarizes the results of this discussion.

Although there is a need for further technology development for leishmaniasis, the working group came to the conclusion that many tools and methodologies already exist. In the course of discussion, it was apparent that many of the IDRC-supported leishmaniasis projects had generated useful and appropriate technologies (Table 2). In most instances, however, these technologies and techniques have not yet been transferred from research institutions to health systems. The working group shared the view that it is important to establish links between these two systems in order to ensure technology transfer. Similarly, the group felt that research networks to foster the exchange of technologies and information would also be an important mechanism for improving the availability and transfer of technologies.

Table 1: Technology Needs, Availability and Transfer for Leishmaniasis

Purpose	Technological Need	Prospective Use and Users	Estimated Time for Readiness
Mass Diagnosis	- Development of clinical screening tools - validation of new tools; gold agg., DAT, DOT-ELISA, improved Leishmanin	Community workers	2 years
Infection Assessment (Humans, vectors, and reservoir)	- Western Blot - DNA probes - Monoclonals - ELISA - PCR (development of other enzymes) - Improved traps (new designs and different attractants (e.g. pheromones, light) - Nets for information and diagnosis - Kits - Educational materials	- Parasite detection in humans, vectors and reservoirs - To collect vectors and reservoirs - Vector population monitoring (indoor/outdoor) - To support taxonomy studies - To detect resistance - Training	- In place 2 years on average

Purpose	Technological Need	Prospective Use and Users	Estimated Time for Readiness
Understanding Reservoir Interaction	Tools for reservoir incrimination	Understanding transmission dynamics in selected foci	2 years
	New approaches to ecoepidemiology (population dynamics)	Improve reservoir incrimination studies	2 years
	Behavioural studies, community surveillance	Future reservoir control	3 years
	Immunogens	Vaccine development	3 years
	Protective devices	Control peridomestic transmission	2 years
Treatment	- Assessment cure PCR - Liposomes - IMMRp	Clinical combination with new drugs	2 years
Training	Development of tools for training	Technical staff, administration, community	?

Table 2: Summary of Tools for Leishmaniasis Generated by IDRC-supported Projects

TOOL	SOURCE	CURRENT STATUS
(a) Clinical Screening Tool	CIDEIM, Colombia	· Detailed documentation published
(b) Leishmanin test	Minas Gerais University, Brazil	· Standardized, high quality, control conditions · Laboratory network currently using it
(c) · PCR for assesement of cure of <u>L. braziliensis</u> · PCR for vector incrimination · PCR for reservoir status assesement	· UPCH, Peru · UPCH, Peru · UPCH, Peru	· Adopted and evaluated · Manuals distributed · Ad-hoc agreements · Standardized · Protocol needs distribution
(d) Education technology adapted to leishmaniasis, for use within context of health education and social communication practices	CIMDER, Colombia	· Needs distribution, adaptation and validation
(e) MBU impregnated cloth	ICIPE, Kenya	· Requires manuals, distribution, adaptation and validation
(f) Rapid epidemiological assesement methodology	U. Nac. Heredia, Costa Rica	· Requires distribution, adaptation and validation

**Working Group #3:
Regional Patterns of Leishmaniasis as a Basis for Action**

This working group had the task of examining those aggregate characteristics of leishmaniasis that are definable into geographic regions, as a basis for prevention/control. The group initially addressed the overall needs for possible regional strategies and then defined the required action for each of the areas of needs (Table 1). The discussion then focused on considering leishmanial species distribution as a possible target for concerted attention (Table 2), with particular emphasis on the individual/community; vectors; and animal reservoirs as intervention points.

Table 1: Generalized Regional Strategies for Control

OBJECTIVE	ACTION
1. Transfer of knowledge and technologies generated by research to national health systems and within the region	Regional workshops, short term training, promoting scientific exchange and consulting
2. Development of standard protocols, sharing of reagents and terms of reference in order to achieve unified criteria and quality control for: - e.g. leishmanin preparation and utilization - monoclonal techniques for parasite identification	Generate manual of biological standards, Create mechanism for production, and distribution within the region in particular.
3. Integration of leishmaniasis control with other vector borne disease control programs	Each country should seek the most appropriate mechanism to achieve integrated control but regional approaches based on knowledge of migration and socio-cultural and geographic factors.
4. Incorporate diagnosis, treatment, and follow up into Primary Health Care (PHC) programs with community participation	Motivate existing health care and community structure representatives to develop plans based on successful models from management of other diseases via PHC

OBJECTIVE	ACTION
5. Expeditious implementation of research findings of epidemiological studies	Operational analysis of risk factor data from previous and ongoing studies in view of possible interventions, including changes in behaviour and environmental management. Carry out further analytical, multidisciplinary epidemiologic studies to identify risk factors in high endemicity foci
6. Simplify and reduce the cost of treatment	Multicenter controlled clinical trials with intralesional antimonial (Glucantime), according to WHO recommendations New trials for other available drugs including BCG/leishmanin using standard and consensus protocols
7. Research on and application of ecological/environmental management for vector control and reservoir control	Identify vector resting sites and ecology in the domicilliary environment in order to reduce contact with man Identify breeding/oviposition sites in order to apply innovative control strategies (e.g. calcium carbonate, biological control, appropriate waste disposal, etc.) Identify modifiable environmental factors that could contribute to transmission control e.g. Type and density of vegetation around and within houses, light and other factors that may modify man/vector contact; ecological methods of rodent control.

Table 2: Leishmanial Parasite Distribution-based Approaches to Control

MAIN PARASITE SPECIES	TARGETED APPROACHES TO CONTROL		
	(a) Community/Individual	(b) Animal Reservoirs	(c) Vectors
<u>L. major</u>	Health education for communities, government directed education on relationships of new settlements, irrigation, mining, agriculture, military, industry Vaccine - BCG/killed vaccine/or combination (development time 2 years ?) Drugs - topical (e.g. Paramomycin) - sub lesional injection	Rodent control - burrow spraying/flooding - burrow destruction	Domiciliary spraying
<u>L. tropica</u>	Community health education Individual protection measures L. major vaccine may provide protection	Reservoir identification	Domiciliary spraying Sanitary measures
<u>L. ethiopica</u>	New drug needed for human reservoir foci	Traps and targetted shooting of Hyrax population	Residual chemical control

MAIN PARASITE SPECIES	TARGETTED APPROACHES TO CONTROL		
	(a) Community/Individual	(b) Animal Reservoirs	(c) Vectors
<u>L. infantum</u>	Relevant training and education of people and health care personnel in transmission patterns involving dogs. Rapid case detection Determination of criteria for cure	Selective dog destruction or diagnosis and legislation for dog control	Domiciliary spraying
<u>L. donovoni</u>	Mass health education, especially in tropical Africa Rapid case detection (e.g. DAT) Rational drug use to counter resistance (e.g. Amphotericin B/ Liposome	Identification of main animal reservoirs	Bednet trials Spraying termite hills House spraying

Working Group #4: Surveillance Systems for Leishmaniasis Control

The working group began its discussion by broadly considering what surveillance tools for leishmaniasis control currently exist. In this, the group considered tools for both active and passive surveillance. It was felt that effective information systems were an important condition for surveillance of any type.

The group was in agreement that surveillance could, and should, exist at various levels of the health care system; that is, at the primary, secondary and tertiary levels. It was acknowledged that surveillance can take several forms. It can be integrated with on-going health care control activities (e.g. with malaria or rabies campaigns, etc) or it can be implemented as an independent program. Independent programs could be vertical or decentralized in their approach. Similarly, the working group spent some time considering the various types of surveillance which could be undertaken. The group identified three general types of surveillance -- qualitative, quantitative and preventative epidemiology.

The group then moved on to summarize the surveillance resources which can be used at the various health care levels as follows:

Community Level (Primary Health Care)

1. Surveillance should be carried out by Primary Health care personnel in the field -
-"Health Promoters" "Health Advisors etc. with the support of:
 - Health volunteers
 - Community leaders
 - Women "Mothers School"
 - Community organizations
2. These should promote community awareness (knowledge of the problem and actions to take) with the aid of all the available media.
3. Diagnostic procedures which can be used (where applicable or feasible):
 - Clinical assessment (emphasizing new lesions and scars CL)
 - Montenegro (Leishmanin skin test) (CL)
 - Parasitological detection by smear (CL)
 - Domestic reservoir detection (taking of blood sample) and selective elimination of positive dogs, when resources are available (VL)
4. Passive case detection would be the standard operating practice. Active case detection to be encouraged where feasible and when needed.

5. Referral of suspect cases to the Secondary Health Care Level

Secondary Health Care Level

1. Diagnostic laboratory/hospital facilities and personnel (laboratory technician, nurse, medical officer etc.)
2. Diagnostic procedures which can be used (where applicable or feasible):
 - Clinical assessment
 - Serology using available means (VL)
 - Parasitological confirmation
 - Montenegro (CL)
3. Information systems:
 - Epidemiological monitoring of the population at risk
 - Dissemination of information
 - Supervision of primary health care workers
 - Training
 - Implementation of control strategies

Tertiary Health Care Level

1. Major Hospitals, Institutes of Higher learning, Advanced research laboratories etc. (Laboratory staff, Physicians, Social scientists, environmentalists etc)
2. Diagnostic procedures which can be used:
 - Data collection analysis
 - Epidemiological design
 - Treatment strategies and management of complicated cases
 - Distribution of technical information
 - Promotion and use of high technology diagnostic tools that could eventually be transferred to other health levels.
 - Coordination of trans-sectorial cooperation
 - Development of strategies for control
 - Promotion and implementation of continuous feedback between all health levels
3. Establishment of an advisory committee for the coordination of surveillance activities at all health care levels.

The working group concluded with the following recommendations:

1. The process of decentralization of health services and integration of control and surveillance activities should be continued. In countries where the resources are not sufficiently available, care should be taken to minimize the disadvantages that may be present in a integrated system where a dominant health problem (e.g. malaria) may undermine the importance of programs to which less priority (e.g. leishmaniasis) is ordinarily assigned. For example consideration can be given to integrating leishmaniasis surveillance activities with those directed at other vector borne diseases or other infectious or parasitic diseases as dictated by local conditions and priorities.
2. Initial surveillance should be implemented universally to establish geographical extension and monitor the level of disease activity. When, based on these semi-quantitative sentinel measures and disease activity seems to be above a critical threshold, active case detection should be implemented to obtain quantitative data. Furthermore, quantitative surveillance activities will be essential to stratify priorities for application of control strategies.
3. Establishment of a mandatory reporting and notification system of leishmaniasis cases at all levels.
4. It is understood that the use of surveillance is essential to the application and monitoring of the effectiveness of the control strategies.
5. To promote the establishment of a field laboratory network at the secondary health level and a multinational laboratory network at the tertiary health level with the principal objectives to standardize laboratory methods and ensure quality control of the reagents.